Flooding Resistance and Life Histories of Short-lived Floodplain Herbs

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FLOODING RESISTANCE AND LIFE HISTORIES OF SHORT-LIVED FLOODPLAIN HERBS

FLOODING RESISTANCE AND LIFE HISTORIES OF SHORT-LIVED FLOODPLAIN HERBS

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van de Natuurwetenschappen

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CONTENTS

VOORWOORD

Wanneer je moeder wordt van twee kinderen ten tijde van je promotie-onderzoek en het schrijven van het proefschrift combineert met de gedeelde zorg voor de kinderen, dan verschijnen er onwillekeurig wel eens visioenen van het leven van promovendi die ontheven zijn van dergelijke taken. Niet dat ik twijfel aan de juistheid van mijn keuze, maar ik ben me in de afgelopen jaren steeds sterker bewust geworden van de afhankelijkheid van vrouwen in deze fase van hun leven van de inzet van hun partner. Was ik helemaal vrijgesteld geweest van gezinstaken, dan had dit proefschrift eerder kunnen verschijnen. Had ik er helemaal alleen voor gestaan, dan zou het vermoedelijk nog niet geschreven zijn.

Een oplossing voor dit dilemma is ons van buitenaf niet gegeven. De mogelijkheden van kinderopvang zijn nog beperkt. Die van ouderschapsverlof zijn groeiende, maar nog lang niet voldoende, ook al wordt er niet door iedereen gebruik van gemaakt. Wanneer echter een toename van het aantal vrouwen op de arbeidsmarkt gewenst is, dan is een grotere inzet van mannen aan het thuisfront een eerste vereiste. Zorg en aandacht voor de kinderen kan maar ten dele worden uitbesteed aan derden en waar mama minder tijd heeft, zal papa er wat vaker voor hen moeten zijn. Mijns inziens is dit niet alleen gunstig voor de ontwikkelingen binnen het gezin, maar ook voor de maatschappelijke verhoudingen. Wellicht gebeurt het dan ook wat vaker dat mannen vertraging oplopen bij het schrijven van hun proefschrift en raken we van het sterk gewortelde idee af dat snel geleverde prestaties ook vanzelfsprekend beter zijn. Meer vrouwen, laatbloeiers, autodidacten en andere vreemde eenden in de academische bijt kunnen helpen de discussie aan te wakkeren en aldus de kwaliteit van onderzoek doen toenemen.

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zijn minst minder netjes de deur uit zijn gegaan. Ook de medewerkers van de afdelingen Illustratie en Fotografie leverden een prestatie door kladwerk om te toveren tot fraaie grafieken. Tot hun spijt hield ik het leuke tekenwerk voor mezelf.

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Ter herinnering aan mijn ouders

CHAPTER 1

GENERAL INTRODUCTION

River forelands along major rivers in The Netherlands are bounded by main dykes, which were built between the 8th and 14th centuries to protect human residences from flooding. These ribbon-like zones up to several kilometres wide, are now all that is left of the active delta system which constituted the geology and geomorphology of vast areas of The Netherlands. There is an increasing awarenes of the importance of their protection as a "nature reserve" and of sensible management of other land use. Parts of forelands are used for minor agricultural activities and grazing as well as for the excavation of minerals. For agricultural purposes, so-called summer dykes were built against flooding in the growing season, which have also become important for certain vegetation types (Van de Steeg 1984). However, in the last decades summer water levels have occasionally even exceeded the heights of these dykes (Van de Steeg 1984; Brock, Van der Velde & Van de Steeg 1987). High water levels are brought about by precipitation peaks in the Rhine catchment area. Due to improved drainage of upstream parts of this area as well as straightening of meanders to facilitate shipping, large quantities of precipitation lead to frequent overflow of the river bed. These unpredictable peaks are superimposed on the more predictable yearly pattern of water discharge by the river, which is relatively high in winter and spring and relatively low in summer and autumn. River forelands are over-dynamic, miniature versions of the large, natural floodplains of rivers which now have largely disappeared in Europe and North America and are quickly disappearing elsewhere (Maltby 1991). Forelands possess "natural" levees, consisting of sandy loam soils, which are typically situated nearer the river bed. Interjacent between these levees and the river, dynamic sandy beaches occur. At greater distances from the river, low mud flats with clay soils are found. Forelands are often intersected by former river beds. Man-made levees are exemplified by the aforementioned summer dykes and man-made depressions are found as numerous sand, clay and gravel pits. As a result of these differences in height, flooding gradients exist.

Several species of the cosmopolitan genus *Rumex* are found in river forelands and each appears to be restricted to a certain zone in the flooding gradient. Among others, this genus was chosen to study adaptations of plants to flooding (Blom 1985; Blom 1990; Blom *et al.* 1990). Two theses have already been published on this subject (Voesenek 1990; Laan 1990). Laan (1990) focussed on differences in flooding tolerance between *Rumex* species from different origins in the flooding gradient. Most of his study dealed with tolerance and mechanisms of alleviation of oxygen deficiency due to flooding in the established phase. Voesenek (1990) compared the flooding resistance of three *Rumex* species from different elevations in relation to their natural habitat. He used the term resistance as the combination of both tolerance and avoidance strategies (*sensu* Levitt 1972). Voesenek stressed the importance of considering flooding regimes as well as integration of all stages of the life cycle in ecophysiological studies (see also Blom 1979). His work covered elements of the regenerative phase of the life cycle and of the vegetative part of the established phase (*sensu* Grime 1979).

Table 1: Flooding characteristics during the period 1971-90 on an old river-bed shore in the Kekerdomse Waard near Nijmegen, The Netherlands. Values of a relatively high site at 9.80 m +NAP (Amsterdam Ordnance Datum) and a relatively low site at 9.30 m +NAP are presented (one number or date concerns both sites).

Year	Time of soil exposure *a*	Flooding characteristics in the growing season *b*				
		Frequency	Duration (days)		Maximum depth (cm)	Time of occurrence
			Min.	Max.		
1971	April	1-2	27-1	27-31	130-170	June
1972	April	3-7	3	9-11	22-72	June-July
1973	April-June	5	6-1	21-18	102-129	April-August
1974	April	2-3	5-3	8-39	22-72	July
1975	May-August	4-1	3-45	41-45	130-140	June-Sept.
1976	April	0-1	0-2	0-2	0-16	August
1977	June-Sept.	5-0	2-0	9-0	48-0	August
1978	April-August	2-1	14-2	89-2	415-16	April-August
1979	July	0-2	0-1	0-2	0-3	August
1980	April-May	3	2	74-99	409-459	April-August
1981	May	4	6-2	20-37	160-210	June-August
1982	April-July	5-1	5-34	38-34	116-118	May-August
1983	July	0-1	0-4	0-4	0-13	September
1984	April	5	1-2	39-58	336-386	May-June
1985	April-July	2-1	13-2	70-2	150-3	May-June
1986	July	1	3-5	3-5	40-90	September
1987	September	3	1-2	6-10	127-177	October
1988	June-July	1-2	4-2	4-17	22-72	October
1989	May	0	0	0	-	-
1990	April	1-3	6-3	6-15	33-83	July

a Theoretical time of exposure: relative river-water level below soil level for at least 7 successive days from April onwards; *b* Flooding characteristics (soil waterlogging and higher water levels) between time of soil exposure and the onset of winter flooding: *i.e.* flooding frequency, minimum and maximum durations, maximum water depth, main time of occurrence; *c* Theoretical end of growing season: time of first severe flooding period from October onwards; *d* Summed number of days of flooding during the winter until exposure in the following year; *e* Month in which peak flooding occurs in the winter; *End of record.

The central theme of the present thesis is how established plants deal with flowering and reproductive development under conditions of highly fluctuating water levels. In contrast to both earlier theses, this study presents a habitat approach to allow the comparison of different strategies. The most frequently flooded areas in river forelands, such as mud flats, shores of old river beds and clay pits were chosen for study. In these areas, floods determine several important events with respect to plant life histories, such as the start and end of the growing season, timing, frequency, duration and depth of inundations during the growing season, and duration and depth of inundations in the winter.

Table 1 continued:

End of growing season *c*	Characteristics of winter flooding		
	Total duration *d* (days)	Maximum depth (cm)	Time of maximum depth *e*
(April 1972)	9-19	18-68	April
November	37-130	333-383	November
December	67-100	249-299	February
October	168-277	396-446	December
November-Dec.	30-61	177-227	January
December	116-240	415-465	February
November	120-284	390-465	March-May
December	163-185	438-488	March
November	102-176	521-571	February
November	131-165	417-467	March
September-Oct.	196-296	484-534	January
October	234-259	545-593	May
December	72-94	498-548	February
November	49-172	296-346	November
January	126-148	384-434	January
October	230-269	445-495	January
November	188-236	553-603	March
December	99-130	331-381	December
December (October)*	48-57	431-481	March

The variability of all these factors is illustrated in Table 1, which summarizes the flooding conditions during the period 1971-90 on the shore of an old river bed of the Waal near Nijmegen. The three consecutive years 1987-89 that were covered by this study were highly different with respect to flooding regime. The first year was extremely wet and several low areas in the river forelands remained flooded until September, leaving a very short growing season. The second year provided a more or less intermediate flooding season, while the third growing season was extremely dry and other factors such as drought stress and predation were probably more important for plant performance during this year than flooding (see Chapter 6).

Two *Rumex* species occur in these low areas of river forelands: *Rumex maritimus* L. (see also Laan 1990) and *Rumex palustris* Sm. (see also Voesenek 1990). In contrast to *R. maritimus*, *R. palustris* remains vegetative in the greenhouse in 16-h photoperiods. Greenhouse studies as wel as field observations (H.M. van de Steeg, unpublished) strongly suggested differences between the life histories of both species, *R. maritimus* being annual or biennial and *R. palustris* being predominantly biennial. In addition to both species of *Rumex*, another representative of the most frequently flooded areas was selected. *Chenopodium rubrum* L. occurs in the same habitat types as both species of *Rumex*, but more frequently on sandy soils (Chapters 2 and 4). The life history of *C. rubrum* is strictly annual and much is known about the flowering requirements and physiology of this species. Early reproduction is essential in C. *rubrum* since survival until the second growing season is impossible (Cumming 1969; Williams 1969; this thesis).

The three species have several characteristics in common which agree with the high disturbance or "loss rates" in their habitats according to traditional and modern theories (e.g. MacArthur & Wilson 1967; Grime 1979; Tilman 1988), such as the occurrence of persistent seed banks, fast growth, relatively short life histories and a high frequency of flowering. In habitats where variations in time and space are unpredictable, plasticity of life-history traits is to be expected rather than genetic variation (Bradshaw 1965; Fitter & Hay 1981). Opposing selective forces are likely to act on species which alternatively grow in air and in water (Arber 1920; Sculthorpe 1967; Ridge 1987). This study investigates plasticity of important life-history characteristics and trade-off patterns in relation to the main effects of flooding presented in Table 1. The prevailing flooding regimes may be experienced as a stress or as a disturbance (*sensu* Grime 1979) according to the longevity of plants (see Menges & Waller 1983). This formed the basis of the main experiments that were performed in outdoor experimental plots in which plants were separately grown in pots that were large enough to allow the development of normal tap roots (Fig. 1). Water levels in these plots could be regulated between drained conditions and complete submergence. Under conditions of high loss rates, resource levels are not likely to be drawn down to levels favouring competition (*e.g.* Harper 1977; Grime 1979; Tilman 1988). Therefore, this design of experiments with plants grown in separate pots seemed justified, provided that results obtained from them are supported by field observations.

Chapters 2 to 7 present the results of studies performed in the experimental plots as well as of greenhouse and field studies. The first to be discussed is a greenhouse study of the effect of flooding regimes (varying time, frequency and duration) on growth and reproductive development in *C. rubrum* and *R. maritimus* (**Chapter 2**). Overall differences in resistance towards partial submergence were found between both species but responses were greatly modified by flooding regimes. In the river forelands, water-level fluctuations are often much more extreme and include periods of total submergence (Table 1). In addition, outdoor conditions are more realistic with respect to the onset of flowering and reproductive development. Therefore, the following chapters consider studies of life-history characteristics in relation to several submergence regimes in the outdoor experimental plots. **Chapter 3** presents a detailed study of flowering phenology and the allocation of dry matter into seeds in *C. rubrum*, *R. maritimus* and *R. palustris* in relation to the time of germination. **Chapter 4** deals with the effect of several flooding periods of mean duration and depth, on survival, growth and reproductive characteristics during the first growing season of all three species. Chapters 3 and 4 together provide an insight in the inter-relationships between the two main effects of flooding in the growing season, *i.e.* delay of germination and disturbance of established plants, and the reproductive development of the three species. **Chapter 5** discusses the role of rapid

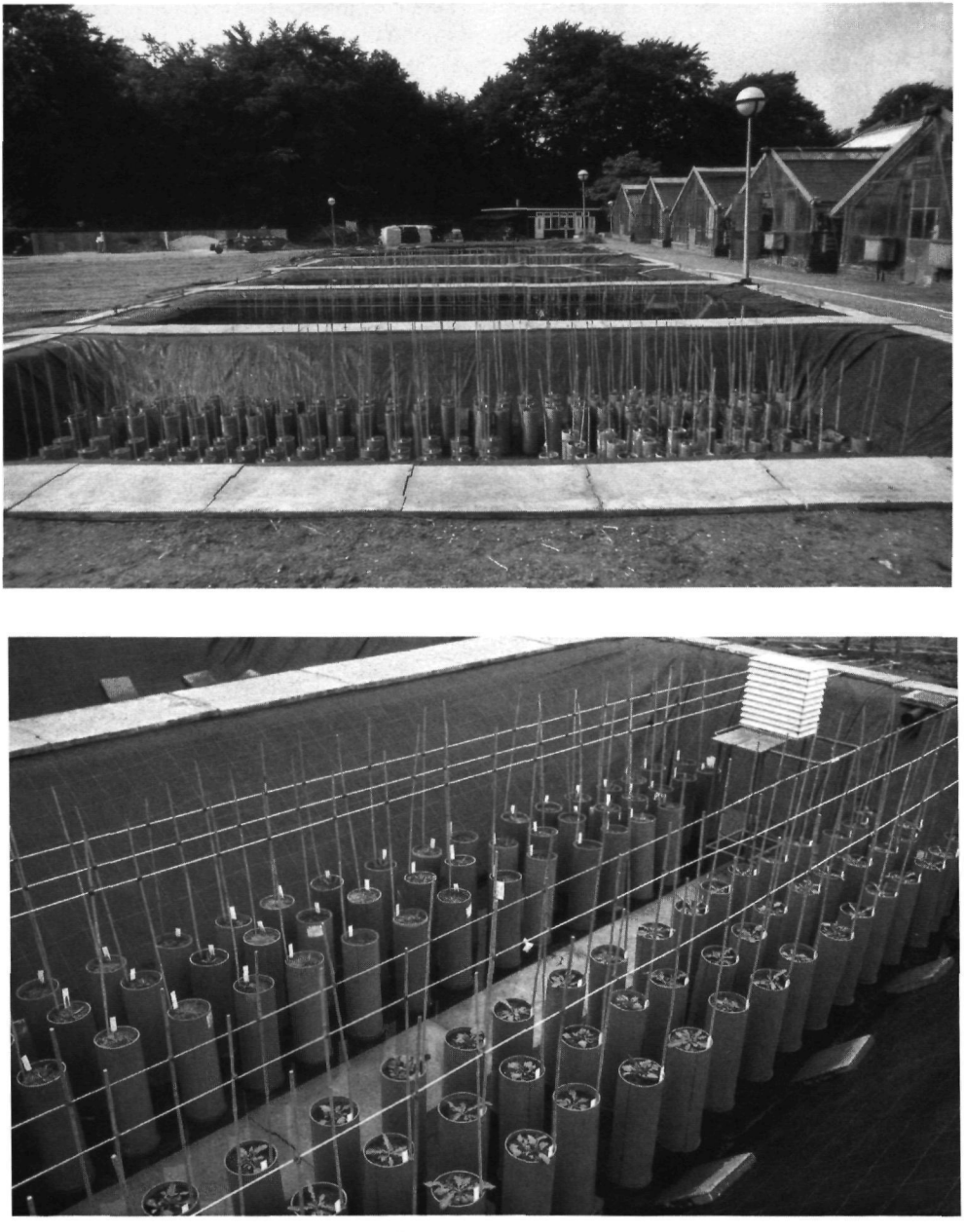

Fig. 1. Experimental set-up of the outdoor experiments, described in Chapters 3-7.

under-water shoot elongation in the ability of both species of *Rumex* to survive and reproduce after more catastrophic periods of flooding in the first growing season which were lethal to *C. rubrum*. This subject was studied in relation to environmental conditions as well as plant developmental stage. In **Chapter 6**, the occurrence of different life histories in *R. maritimus* and *R. palustris* in river forelands is discussed in relation to their strategies to cope with flooding. Also in this chapter, attention is paid to the role of under-water shoot elongation in early spring for survival of prolonged winter flooding and reproductive development in the following growing season. The hormonal regulation of the rapid under-water shoot elongation responses which are presented in Chapters 4-6 is discussed in **Chapter 7**. This chapter considers a physiological study of ethylene accumulation and production in relation to elongation responses in different developmental stages in *R. maritimus*. **Chapter 8** provides a general discussion of variation in relevant life-history traits in relation to flooding conditions. Avoidance and tolerance strategies were distinguished in all three species, but on different levels of organization.

CHAPTER 2

GROWTH AND REPRODUCTION OF RUMEX MARITIMUS AND CHENOPODIUM RUBRUM UNDER DIFFERENT WATERLOGGING REGIMES

with O.F.R. van Tongeren and C.W.P.M. Blom

The influence of different waterlogging treatments on shoot development and subsequent flowering and seed production of plants of *Chenopodium rubrum* and *Rumex maritimus* was studied in a greenhouse experiment.
C. rubrum, a typical occupant of low sandy beaches at times when floods have subsided, appeared to be suppressed by all waterlogging regimes. *R. maritimus*, an inhabitor of the wet mud flats of old river beds, was found to be quite tolerant to waterlogging conditions; early waterlogging, during the rosette stage, led to an increase in dry weight and seed production in some cases. In general, seed production changed more than biomass as a result of waterlogging, but responses varied in accordance with flooding regimes. Intermittent waterlogging generally caused more damage than did continuous waterlogging, especially in *C. rubrum*. Even in *R. maritimus*, seed production was reduced under conditions of intermittent waterlogging.
The results demonstrate that adaptations to, and damage from, flooding greatly depend on flooding regimes which vary substantially in the field situation.

Introduction

During the last few decades river forelands of the Rhine-branches in The Netherlands were flooded more frequently in summer, due to a faster rain-water discharge from the upper Rhine-basin into the river. As a consequence, plants were subjected to more frequent and earlier floodings of longer durations, which has a strong influence on flora and vegetation (Van de Steeg 1984; Brock, Van der Velde & Van de Steeg 1987). Specific morphological responses upon waterlogging under controlled conditions are chlorosis and accelerated senescence of leaves (Jackson & Campbell 1975, 1976; Trought & Drew 1980a). There is often an accompanying reduction in growth of above-ground parts and withering of the root-system (Kramer 1951; Jones & Etherington 1971). Quite common effects of waterlogging are the formation of adventitious roots and of aerenchyma in these roots and in other plant parts (Iversen 1949; Armstrong 1975, 1978; Etherington 1984; Schat 1984). Regrowth of above-ground parts frequently occurs after adventitious roots are initiated. Jackson (1955) found that these roots have a positive influence on the recovery of some species during waterlogging.

The present study is part of a project aimed at studying the effects of flooding on ecophysiological and population characteristics of some plant species which occur in the marshes along rivers in The Netherlands (Voesenek & Blom 1987; Voesenek, Blom & Pouwels 1989). Our study concerned a greenhouse experiment on growth and reproduction in two annual species which are common in the Dutch riverine area, *i.e. Chenopodium rubrum* L. and *Rumex maritimus* L. Both species occupy habitats that are irregularly flooded in summer. *C. rubrum* is a common species of sandy beaches, adjacent to the river. To a lesser extent this species occupies the mud flats of former river beds, where *R. maritimus* has its optimal occurrence (Salisbury 1942, Tüxen 1979). In these more anaerobic habitats only small individuals of *C. rubrum* are found. Both species are frequently found as pioneer species in clay and or sand pits

as well, which are quite common habitats in the Dutch riverine area. Germination of both species can occur from the end of May until late summer, after the floods recede. Plants of *R. maritimus*, which germinate late in the season, will probably not flower the same year but winter as a rosette. The second year flowering occurs unless rosettes succumb to winter flooding (Hejny 1960). Plants of *C. rubrum* which germinate early in the season grow vegetatively during the summer and can become quite large, whereas late germinating plants remain small; all plants flower late in the summer under short daylight conditions (Cumming 1969; Bernier, Kinet & Sachs 1981a). The water regime seems to be an important environmental factor for vegetative and generative development in both species and was, therefore, chosen to be studied in detail in a greenhouse experiment.

Materials and methods

Main growth experiment

Plants of *C. rubrum* and *R. maritimus* were grown from seeds which were 2-year-old and dry-stored after being collected in the Kekerdomse Waard near Nijmegen, The Netherlands. Uniform germination was achieved in petri dishes in a growth chamber under a fluctuating temperature regime of 30 °C and 15 °C during the 12/12 h photo- and dark-period respectively. The young seedlings were transferred immediately to the greenhouse, where they were transplanted into plastic containers (diameter 18 cm), that were filled with an industrial peat (Jongkind No.5). The photoperiod was kept at 16 h by means of 400-W high-pressure sodium lamps, which supplemented the normal daylight with a light intensity (PAR) of 110 $\mu Em^{-2}s^{-1}$ at plant level (minimum during the periods without daylight).

The main experiment was carried out to study the effects of different waterlogging regimes on growth and reproduction. An additional series of plants was used to obtain extra information about morphological changes and to acquire biomass estimates for growth curves of individual plants in the experiment (see next paragraph).

The experiment was started in the period of exponential plant growth, 6 weeks after sowing. Mean height of the plants of *C. rubrum* was 14 cm; plants of *R. maritimus* were still in the rosette stage, with rosettes nearly fully expanded. During the following 8 weeks, eighty plants per species were subjected to different waterlogging treatments in a completely randomized full factorial design. The different factors of waterlogging that were tested, were duration (0, 2, 4 and 6 weeks of waterlogging, either in one extended period or split into shorter periods), frequency ("continuous", *i.e.* waterlogging in one extended period and "intermittent", *i.e.* waterlogging in four equal shorter periods) and time course ("early", *i.e.* waterlogging beginning 6 weeks after sowing and "late", *i.e.* waterlogging ending 14 weeks after sowing). A scheme for the different treatments is presented in Fig. 1. Per treatment five replicates were used.

Non-waterlogged plants were kept under constant moisture conditions (60% by weight), later referred to as standard watering conditions. Waterlogging was achieved by placing the pots in tanks in which the water level was kept 14 cm above the soil level. All waterlogged plants were completely submerged at the start of the experiment. From 14 weeks onwards all surviving plants were kept under standard watering conditions until flowering and seed formation occurred.

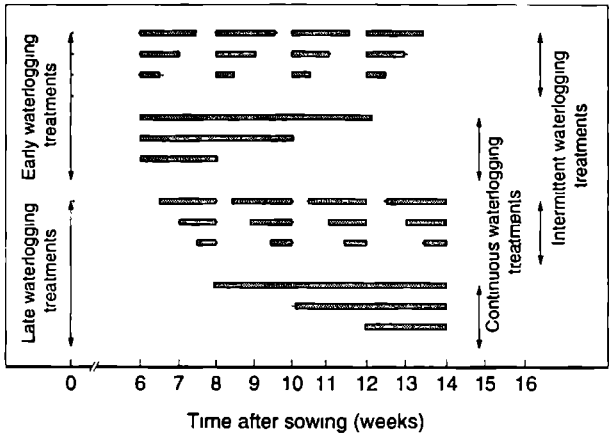

Fig. 1. Scheme representing the waterlogging treatments of the full factorial experiment; Shaded blocks indicate waterlogging periods.

The biomass of individual plants was estimated from stem length and maximum leaf length twice weekly. At the end of the different waterlogging treatments (14 weeks after sowing), the biomass of all plants of *R. maritimus* was estimated. The regression method for estimating biomass, however, appeared not to be accurate in *C. rubrum*. Regression parameters were found to be different for different treatments (see next paragraph for the method by means of which this was tested). Therefore, two plants of *C. rubrum* per treatment were harvested after 14 weeks to obtain more precise biomass values. The remaining plants (three per treatment) were kept to provide the data on seed production. The timing of flowering was determined by recording the first appearance of flower buds. Seeds were collected per plant, dry stored for a few weeks and weighed (perianths included). Several samples were counted to determine whether differences between treatments were due to seed numbers or seed sizes.

Additional measurements

In an earlier study of these species (A.J.M. van der Sman, unpublished), performed under standard watering conditions, biomass could be estimated by the formula:

$$\log(\text{dry weight}) = a + b\{\log(\text{stem length})\} + c\{\log(\text{maximum leaf length})\}.$$

We did not, however, know whether such an equation could be applied to waterlogged plants of both species as well. Therefore, additional plants were grown and placed at random between the plants from the main experiment. Three more extreme treatments were applied to these plants: (i) standard watering conditions, (ii) continuous waterlogging from 6 weeks onwards, and (iii) intermittent waterlogging (alternate weeks) from 6 weeks onwards. To obtain biomass estimates for the plants in the experiment, each week, in the period between the sixth and fourteenth week after sowing, a plant was harvested from each treatment series. Multiple linear regression was performed (by means of the least squares method) on the thirty harvested plants per species with biomass as the dependent variable and stem length and maximum leaf length as independent variables. To distinguish between the treatments two dummy variables were introduced, representing both the waterlogging treatments. When either of these variables showed a significant effect on biomass, the regression equation was modified accordingly. All tests were

performed using the General Linear Models (GLM) procedure from the SAS-package (SAS Institute Inc. 1985).

In addition, number of leaves, number and length of shoots and dry weight were determined at harvest. In *R. maritimus*, mean petiole length of rosette leaves and stem diameter were also measured.

Root performance of the harvested plants was examined for degeneration of the root system, and the formation of adventitious roots and aerenchyma. Specific gravity of roots, measured with a pycnometer (Iversen 1949; Schat 1984), was used to indicate root porosity.

Five plants per treatment were kept waterlogged under the same regimes after the fourteenth week to study the effects on reproduction of prolonged continuous and intermittent waterlogging conditions throughout flowering and seed production.

Results

The first remarkable response of both species upon waterlogging was the change of leaves and stems from a prostrate to a more vertical position. In all cases this was noticed within a few days. Differences in response between the two species were apparent shortly afterwards (Tables 1 and 2).

Waterlogging in *C. rubrum* caused severe chlorosis and shedding of most of the underwater leaves. Stems became weak and started to display strange curvatures; newly formed leaves remained small. Table 1 summarizes some measured above-ground parameters on harvested plants from this species. Growth of most above-ground parts of plants of *R. maritimus*, on the other hand, was stimulated under conditions of waterlogging (Table 2) and, by means of petiole elongation, rosette leaves were being restored to the water surface, resulting in less chlorosis and leaf shedding.

Table 1. Influence of continuous and intermittent waterlogging (alternate weeks) from 6 weeks onwards on various shoot parameters in *Chenopodium rubrum*. Values represent the mean (±1SE) of five plants, harvested between 10 and 14 weeks after sowing.

	Standard watering conditions	Intermittently waterlogged	Continuously waterlogged
Number of leaves > 1 mm/plant	1100±100	400±100	170±50
Maximum leaf length (cm)	10±1	8±1	6±1
Number of branches	15±2	10±1	9±2
Stem length (cm)	51±4	33±1	43±6
Length of first branch (cm)	39±4	23±3	26±5
Dry weight (g)	23±2	5±1	5±1

Table 2. Influence of continuous and intermittent waterlogging (alternate weeks) from 6 weeks onwards on various shoot parameters in *Rumex maritimus*. Values represent the mean (±1SE) of four plants, harvested between 11 and 14 weeks after sowing.

	Standard watering conditions	Intermittently waterlogged	Continuously waterlogged
Number of leaves > 1 mm/plant	220±70	190±50	170±30
Maximum leaf length (cm)	30±2	30±3	39±3
Stem length (cm)	100±8	116±9	135±6
Stem diameter (cm)	1.30±0.05	1.50±0.05	2.2±0.1
Petiole length (cm)*	8.7±0.8	14.5±0.8	14.7±0.6
Dry weight (g)	24±4	21±2	28±4

*Mean petiole length of the five largest rosette leaves per plant.

Table 3. Parameter estimates (±1SE) for the equation $\log(\text{biomass})=a+b\{\log(\text{L.L.})\}+c\{\log(\text{S.L.})\}$* with all 30 harvested plants of both *Chenopodium rubrum* and *Rumex maritimus*.

Parameter	*C. rubrum*			*R. maritimus*		
	Estimate	*F*	*p*<	Estimate	*F*	*p*<
a	-7.7±1.9			-0.9±3.7		
b	1.7±0.4	14.40	0.001	1.4±0.7	4.47	0.05
c	1.5±0.2	47.82	0.001	0.4±0.1	35.24	0.001

*Biomass in mg and leaf length (L.L.) and stem length (S.L.) in mm.

Results of regression analysis with the thirty harvested plants per species are summarized in Table 3. Figure 2 presents growth curves, based on estimated biomass values, which separate the three different factors of waterlogging and the most remarkable interaction term: frequency x time course. The results of both biomass and seed production after 14 weeks are presented for each individual treatment in Fig. 3 (biomass values are from harvested plants in *C. rubrum*). Table 4 summarizes the statistical significance of these results. It is apparent that in both species growth and seed production were significantly influenced by the different treatment factors.

A more detailed examination of the growth curves and the results of the analysis of variance on biomass after 14 weeks indicates that *C. rubrum* suffered from waterlogging, whereas in *R. maritimus* biomass even seemed to be slightly increased by prolonged waterlogging (main effects of duration and frequency). Repeated waterlogging in short periods damaged *C. rubrum* but not *R. maritimus*. This effect was found as an interaction effect, indicating that longer, repeated inundations can be more damaging than the same number of days inundation in one prolonged period. This is, however, dependent on the time of waterlogging. Plants of *C. rubrum* which were older at the time of the first inundation, were damaged less (main effect of time). This effect can also be seen in *R. maritimus* (Fig. 3) but is not significant for biomass values (Table 4). The interaction term duration x time course indicates that the effect of longer periods

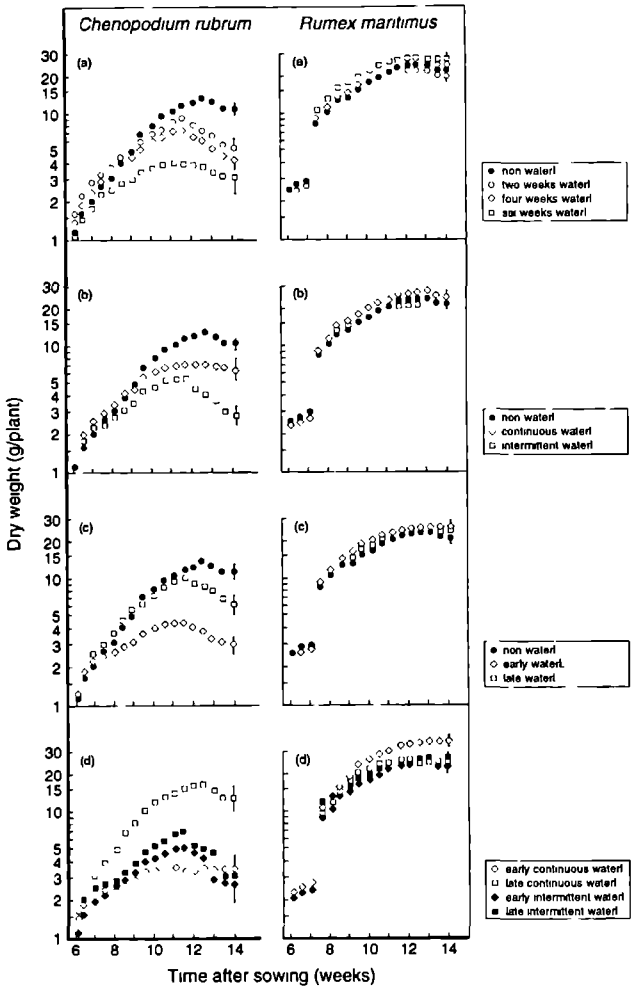

Fig. 2. Growth curves of plants of *Chenopodium rubrum* and *Rumex maritimus*, representing the main effects of: (a) duration; (b) frequency; (c) time; and (d) the interaction frequency x time of waterlogging in the period between 6 and 14 weeks after sowing. See Fig. 1 for the different applied treatments.

of waterlogging was larger in younger plants of *C. rubrum* than in older ones. Frequency x time course interactions in both species indicate that younger plants were less capable of coping with frequently changing conditions than older ones.

The effects of waterlogging on flowering and seed production were even more pronounced. All surviving plants of *C. rubrum* flowered, but the time of flowering was influenced by the treatments. Flowering was delayed in relation to the frequency and duration of waterlogging (Table 5). A delay of more than 2 months was caused by prolonged waterlogging throughout flowering and seed formation. All plants of *R. maritimus* flowered between 13 and 15 weeks after sowing and differences in the time of flowering did not appear to be related to the waterlogging treatments.

All factors of waterlogging had a significant effect on seed production in *R. maritimus* (Table 4). Seed production was increased by continuous waterlogging but decreased as a result of intermittent waterlogging. The significant twofold interaction terms in Table 4 are of special interest. The effects of duration and frequency were much larger in the case of early waterlogging, as can be seen in Fig. 3.

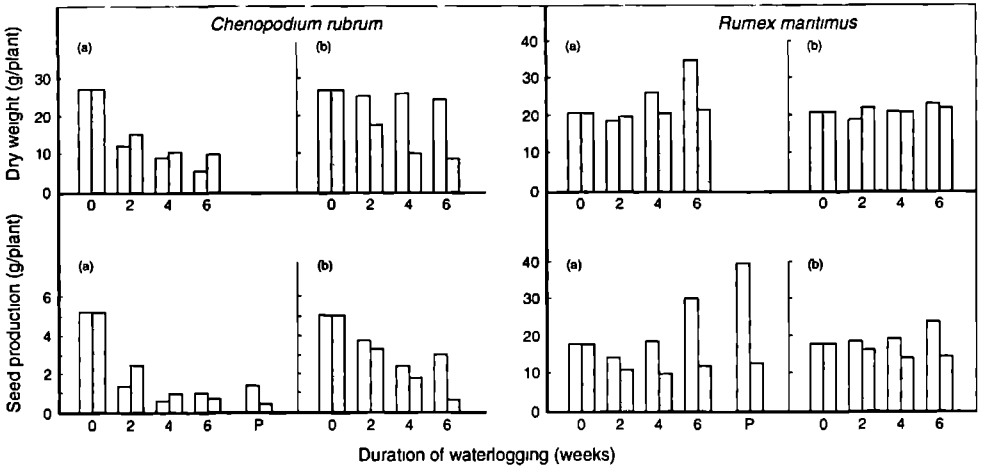

Fig. 3. Effect of waterlogging on biomass and seed production in *Chenopodium rubrum* and *Rumex maritimus*. Horizontal axis: total duration of waterlogging (duration=p[additional experiment]: prolonged waterlogging troughout flowering and seed formation); (a) early waterlogging; (b) late waterlogging; open bars, continuous waterlogging; shaded bars, intermittent waterlogging. See Fig. 1 for the different applied treatments in the main experiment.

Table 4. Statistical significance of the results from the main experiment presented in Fig. 3, separating the effects of different factors of waterlogging on dry weight and seed production in *Chenopodium rubrum* and *Rumex maritimus*. Analysis of variance performed on log-transformed values.

Factor of waterlogging	*C rubrum*		*R maritimus*	
	Biomass	Seed	Biomass	Seed
Duration	***	***	***	***
Frequency	NS	NS	NS	***
Duration x frequency	*	NS	NS	***
Time course	***	*	NS	**
Duration x time course	**	NS	NS	**
Frequency x time course	***	NS	*	*
Duration x frequency x time c.	**	NS	NS	NS

Statistical significance: NS=not significant; *p<0.05; **p<0.01; ***p<0.001.

Table 5. Flowering in *Chenopodium rubrum* in relation to waterlogging. See Fig. 1 for the different applied treatments.

Duration of waterlogging (weeks)				Week number in which the first-last plant per treatment started to flower after sowing*
Intermittent		Continuous		
Early	Late	Early	Late	
2				13-16
			2	14-18
		2		14-18
4				15-18
			4	15-18
6				14-21
	2			15-21
	4			14-23
		4		15-23
	6			19-21
			6	23-25
		6		26-27

*Non-waterlogged plants started to flower 14-18 weeks after sowing.

Seed production in *C. rubrum* was severely reduced because of waterlogging (Fig. 3), but only duration and time course (as distinguishable factors) showed significant effects (Table 4). The data on seed production of the ten additional plants per species which experienced prolonged periods of waterlogging, confirm the above results (Fig. 3). These data show the tendency of adaptation to prolonged waterlogging conditions in both species. Possible mechanisms to avoid the less preferable conditions during waterlogging are found in the additional information on above-ground parameters and the root system that was obtained from the plants which were harvested for regresssion analysis. While growth of leaves and stems was inhibited in *C. rubrum* (Table 1), the stem and petiole lengths of plants of *R. maritimus* increased during waterlogging (Table 2).

After a few weeks of waterlogging plants of *R. maritimus* developed a secondary root system below the soil surface. This consisted of very white and thick roots. Within 5 days of waterlogging, adventitious roots were initiated above-ground. These roots possessed tiny laterals over the entire root length. A few weeks later a mat of roots was formed over the soil surface; these could attain a length of about 30 cm.

As soon as stem growth advanced (*c.* 8 weeks after sowing), adventitious roots without secondary laterals started to grow from nodes just under the water level. Plants which were waterlogged after stems were initiated, developed this latter root type only. Ater drained conditions were restored, most adventitious roots dried out; some of them survived by growing into the soil. Intermittently waterlogged plants, therefore, were unable to produce as many adventitious roots as continuously waterlogged ones.

The specific gravity of primary roots decreased from 1.00 (±0.01) g cm^{-3}, under non-waterlogged conditions to 0.95 (±0.01) g cm^{-3} under waterlogged conditions. The specific gravity of the adventitious roots in the rosette stage was 0.93 (±0.01) g cm^{-3} and of the adventitious roots on the stems 0.85 (±0.01) g cm^{-3}. Light microscope studies of sections showed that most aerenchymatic tissue was located in the latter root type. Aerenchyma developed in the shoots as well and occurred together with an increase in stem diameter (Table 2).

The tap-root system, with delicate branching side roots in *C. rubrum*, disappeared during both intermittent and continuous waterlogging. Root degeneration, starting at the bottom of the containers, was complete after 4 weeks of waterlogging. Under intermittent waterlogging conditions a secondary root system, consisting of relatively thick and unbranched roots, was initiated just below the soil surface. A comparable secondary root system was formed when drained conditions were restored after a long period of continuous waterlogging. After 2 weeks of waterlogging adventitious roots developed from the submerged 14 cm of the stem. On intermittently waterlogged plants adventitious roots remained small (mm); on continuously waterlogged plants they attained a length of 5-10 cm. Primordia of these roots were visible on some non-waterlogged plants as well, but in much smaller numbers.

The specific gravity of all different root types of *C. rubrum* was about 1.00 (±0.01) g cm^{-3}. Microscopic examination of cross-sections revealed some aerenchymatic tissue in the adventitious roots on the stems only. A few plants which remained completely submerged did not produce secondary roots at all. After some weeks these plants died. In both species a stimulation of stem growth was observed shortly after initiation of adventitious roots.

Discussion

Growth and reproduction in both *R. maritimus* and *C. rubrum* were strongly correlated with waterlogging treatments. The change of growth pattern in *C. rubrum* was such that the previously developed method for estimating biomass was not suited for waterlogged and non-waterlogged plants together. Results were, however, little influenced by this bias (it resulted in biomasses estimated a little too large for continuously waterlogged plants compared with other treatments and, therefore, reduced the observed effects rather than enhanced them) and thus growth curves remained quite reliable. The two species differed considerably in their responses to waterlogging. Both species showed the response of reorientation of leaves and stems to a more vertical position, but in *R. maritimus* this response was accompanied by an increase in petiole length (Table 4) until the leaf bases of most rosette leaves reached the water table. This phenomenon has been observed in the field and in our laboratory on other *Rumex* species as well (Voesenek, Blom & Pouwels 1989).

The role of ethylene in this process as well as in stem elongation is studied at present (see Van der Sman *et al.* 1991, Chapter 7 in this thesis). The effect of plant age will be taken into account in future studies, since young plants of *R. maritimus* showed growth stimulation by waterlogging. In *R. maritimus*, most rosette leaves were kept above the water table and only a few leaves developed chlorosis and died. In *C. rubrum*, no such mechanism was noticed and chlorosis of leaves of waterlogged plants was severe at all times and, therefore, leaf senescence was high as well. A few plants which were waterlogged from 6 weeks onwards did not survive

the longest duration of waterlogging. Both in this study and in other studies (Kramer 1951; Jackson & Campbell 1975), chlorosis was correlated with growth reduction of the above-ground parts and the root system. Shoot biomass of small plants of *C. rubrum* was severely reduced in response to waterlogging, whereas biomass of plants of *R. maritimus* remained nearly unchanged. Chlorosis of plants of *C. rubrum* which were waterlogged in a later stage was less severe and correspondingly biomass did not differ significantly from the non-waterlogged plants.

According to Drew (1983), chlorosis and leaf senescence during waterlogging may be caused by: (i) the accumulation of toxins, originating in the soil or in anaerobic roots, (ii) mineral nutrient deficiencies caused by a decrease in energy-dependent ion transport in the roots, and (iii) changes in phytohormone production by the roots. The second possibility is well studied in wheat (Drew & Sisworo 1977; Drew, Sisworo & Saker 1979; Trought & Drew 1980a,b). These studies support the view that, especially in young plants with limited reserves, nitrogen deficiency is an early consequence of waterlogging and causes premature leaf senescence while mobile nutrients are reallocated from older leaves into younger ones. As a result of oxygen deprivation of the roots, nutrient uptake can only take place passively, by mass flow (Drew & Sisworo 1977; Trought & Drew 1980b). The primary root system degenerated far more in *C. rubrum* than in *R. maritimus*. After 6 weeks of waterlogging, plants of the former species lost contact with the soil and started floating in the tanks. In *R. maritimus*, the root system did not appear to change very much under conditions of intermittent waterlogging. Under conditions of continuous waterlogging new roots without secondary laterals were initiated. Plants of *C. rubrum* developed fewer of these new roots and under conditions of intermittent waterlogging only. *R. maritimus* also appeared to be capable of quick and abundant adventitious root formation (see also Laan *et al.* 1989). Tiny adventitious roots, containing less aerenchyma, were formed by larger plants of *C. rubrum* only and after a longer period of time, compared with *R. maritimus*. Adventitious rooting in *C. rubrum* resembles the adventitious rooting pattern in *Epilobium hirsutum* (Etherington 1984).

The more rapid decay of the primary roots in *C. rubrum* in comparison with *R. maritimus*, as well as the lack of aerenchyma in the less abundant adventitious roots, may thus be the cause of heavier chlorosis and faster leaf senescence in this species, acting by means of both toxicity and nutrient deficiency. The latter mechanism may also account for the heavier chlorosis of younger plants of *C. rubrum*, which possess less nutrient reserves than older ones.

R. maritimus appears to be a species which is able to continue aerobic metabolism quite well under waterlogged conditions (see Laan 1990), probably by means of oxygen diffusion from above-ground parts into the root-system (Armstrong 1978). In our experiment, as soon as adventitious roots became visible, an acceleration in stem growth was measured in plants of both species, which demonstrates the importance of these roots (Kramer 1951, Etherington 1984). The important role of adventitious roots can also be inferred from the more damaging effects of intermittent waterlogging that prevented the survival of these roots in both the species, and also the death of plants of *C. rubrum* which were unable to produce them.

Reproduction appeared to be even more distinctly influenced than biomass by waterlogging. Flowering in *C. rubrum* was delayed up to several months due to continuous waterlogging treatments. Seed production was reduced in this species because of all waterlogging treatments, whereas seed production in *R. maritimus* was decreased as a result of early intermittent waterlogging treatments only. All differences in seed production could be ascribed to differences in seed numbers.

In conclusion, *R. maritimus* appeared to be well adapted to waterlogging which is in accordance with its occurrence on former river-bed soils with a low oxygen status. In contrast, *C. rubrum* seemed to be damaged easily by waterlogging, which may be one of the factors that determine the lack of abundant and tall specimens of this species in those sites.

However, the responses in both species varied in accordance with the applied regimes. Waterlogging in the early stages had the most remarkable stimulating effect on stem and leaf growth and resulted in the largest seed output in *R. maritimus*. On the other hand, early waterlogging appeared to be particularly harmful in *C. rubrum*. A large fluctuation of the water level appears to be relatively damaging for both species.

Therefore, the number of days of flooding per season is not the only factor that needs to be registered in the field. Equally important factors are time (with respect to the life-stages of plant species) and the frequency of flooding during the growing season. Another, and equally important factor, will be the occurrence of periods of complete plant submergence, including winter inundations.

The following chapters include field studies as well as experiments on the physiological mechanisms that are involved in the adaptive processes in relation to waterlogging and submergence.

Acknowledgments - We would like to thank Truus van der Mast for help with the experiments and Dr. R. Gulati for correcting an early version of the manuscript.

PHENOLOGY AND SEED PRODUCTION IN CHENOPODIUM RUBRUM, RUMEX MARITIMUS AND RUMEX PALUSTRIS AS RELATED TO PHOTOPERIOD IN RIVER FORELANDS

with C.W.P.M. Blom and H.M. van de Steeg

Reproductive development in three species from irregularly flooded areas of river forelands was studied in relation to time of emergence. In *Chenopodium rubrum*, flowering was induced earlier in plants germinated in April-May than in later cohorts. However, the period of vegetative growth diminished and the life-cycle was completed in a shorter time in later germinated plants. Seed number was reduced, but seed size as well as reproductive effort per plant increased in later cohorts.
Plants of both *Rumex* species flowered after a certain number of leaves had developed and before a "critical" photoperiod had passed. In earlier cohorts, the main shoot and several axillary shoots elongated and flowered. Fewer axillary shoots flowered closer to the critical photoperiod and this resulted in a reduced seed output in later cohorts. The critical photoperiod as well as the time needed for completion of the life cycle was longer in *Rumex palustris* than in *Rumex maritimus*.
It is argued that in the riparian habitat, plants of both *Rumex* species are only occasionally able to complete their life cycle in one growing season. Survival of these species on the population level will rely more upon adaptations towards flooding during the established phase than is the case for *C. rubrum*.

Introduction

River forelands, stretching from the river bank to the main dyke, provide one of the most uncertain habitats for plants (Van de Steeg 1984; Brock, Van der Velde & Van de Steeg 1987; Blom 1990; Blom *et al.* 1990). A striking aspect of flooding in these areas is the variation in the length of the growing season. Elevational differences of a few decimetres can cause the growing season to vary by a month or more (Chapter 1). Successive years differ to such an extent that the lowest areas may dry out in April or remain flooded until as late as September. Survival of populations of annual and biennial plants depends upon successful regeneration by seed, and this process involves a number of important life-history traits that are greatly affected by limiting factors such as a short growing season (Grime 1979).

This paper discusses flowering phenology and seed output in the first season of growth for three representative species from the lower elevations in river forelands. These life-history phenomena are compared for plants which germinated at different times during the growing season. Red goosefoot, *Chenopodium rubrum* L., and the two dock species *Rumex maritimus* L. and *Rumex palustris* Sm. cohabit the mud flats of former river beds and, both clay and sand-pits (see Salisbury 1942; Hejny 1960; Van der Sman, Van Tongeren & Blom 1988, Chapter 2 in this thesis; Voesenek 1990). Under ruderal conditions, the greatest emergence of *C. rubrum* is in May-June (Williams 1969; Roberts & Neilson 1980), and that for *R. maritimus* is in April-May (Roberts & Boddrell 1985). All three species show a maximum germination response under a combination of fluctuating temperatures and light (Cumming 1959; Voesenek 1990; Van der Sman, Joosten & Blom 1993, Chapter 4 in this thesis), which is common for wetland species. This response provides a mechanism whereby spring germination is initiated by an increase in irradiance and a falling water table (Thompson & Grime 1983). Because germination is

inhibited under strictly anaerobic conditions in all three species (Voesenek 1990; A.J.M. van der Sman, unpublished), germination on the river forelands depends upon the subsidence of winter and/or spring floods. Reproductive development was studied in outdoor experimental plots for three consecutive years. The performance of the short-day annual *C. rubrum* in these experiments can be explained by the well-known flowering physiology of this species and this is in accordance with general field observations. Little is known, however, of the flowering requirements of both *Rumex* species. Field observations of these species are less easily interpreted, since plants may be annual, biennial or even short-lived perennial (Chapter 6). Therefore, in both *Rumex* species, the effect of photoperiod on flowering behaviour was also studied, and a field study of their phenology in two sites in the riparian habitat is presented.

Materials and methods

Seeds of *C. rubrum*, *R. maritimus* and *R. palustris* were collected from individual plants in the Kekerdomse Waard near Nijmegen in the year preceding each experiment and then stored dry at room temperature. After removal of perianths (*Rumex* spp.), a mixed seed sample from five different parent plants of each species was imbibed (12 h light 25 °C/12 h dark 10 °C; mean photosynthetically active radiation (PAR)(400-700 nm) during the light period was 30 μmol m^{-2} s^{-1}). After 4 days, five to seven seedlings were transplanted into individual pots (diameter 16 cm, height 50 cm in outdoor experimental plots; 10-cm pots in photoperiod experiment) with perforated bases. Each pot was filled with a 1:1 (v:v) mixture of river sand and peat. Seedlings were thinned to one per pot within 2 weeks.

Phenology in outdoor experimental plots

To study reproductive development in relation to time of emergence, plants were grown in cohorts for three consecutive years, *i.e.* 1987-89, inclusive. Immediately after transplantation of each cohort, the pots were placed, 16 cm apart, in outdoor basins of water (length x width x depth: 8 x 2.4 x 1 m; water level 5 cm). During extremely dry periods, young seedlings were watered by sprinkling to prevent desiccation. The soil-moisture content in the pots averaged 31% by weight (*c.* 24% in the upper 10-cm and *c.* 38% in the lower 10-cm soil layer). Daily radiation and temperatures were recorded throughout the course of the study period. The latter are presented as temperature sum, *i.e.* the number of degree-days over a certain period with 10 °C as base temperature. This base was chosen because none of the species showed significant growth at lower temperatures.

In 1987, four cohorts (each of twenty plants per species) were planted at 6-week intervals, on 18 May, 29 June, 10 August, and 21 September. This was designed to approximate the extremes in length of the growing season. Only the plants of the first cohort of *R. palustris* flowered and those of the first and second cohorts of *R. maritimus*. Both flowering cohorts of *R. maritimus* showed marked differences in flowering characteristics. Therefore, for both *Rumex* species, six cohorts (twelve plants per species per cohort) were planted in the following year. Two-week intervals separated each cohort, which commenced on 2 May and ended on 11 July. In the third year, seven cohorts (twelve plants per species per cohort) of all three species were planted at 1-week intervals, starting from 10 April until 22 May. The latter was designed to study

reproductive development with respect to the length of the growing season under the most favourable conditions of spring germination.

Plant measurements

Vegetative growth of rosettes in both species of *Rumex* was measured by recording the number of leaves > 1 cm long, the number of dead leaves, and the length of the longest leaf (including petiole) of the main shoot. The number of axillary shoots and the number of living leaves per shoot were counted approximately every 10 days. The same parameters were measured in *C. rubrum*, but including the lengths of the main stem and of branches (longer than 1 cm). The transition to the generative phase was recorded. Several stages were distinguished, *i.e.* bolting (*Rumex* spp.), appearance of clearly visible flowering buds (*C. rubrum*), duration of flowering, and ripening of fruits. Dates were recorded per plant for these parameters; 50 % values per cohort are presented in the results section. The numbers and final lengths of flowering shoots were measured in both *Rumex* species. Seeds with perianths were harvested from each sample plant and for each of the three species. All seed samples were sieved to remove dead leaves and sand and then weighed to estimate total seed production per plant. Per cohort, the total seed output of five plants was mixed. From these mixtures, two x four subsamples of fifty seeds were weighed to estimate individual seed weights. These samples were also used to determine germination response. After the seed harvest, the stems were dried (48 h at 70 °C) and weighed. In 1989, seeds were harvested from five plants per cohort. Total above-ground weights (including seeds) were determined and seed weight estimated (from the ratio between seed weight and weight of all other above-ground parts of the first five plants) for the remaining seven plants. In December 1987, four plants per species were harvested from all cohorts to determine dry weights (48 h at 70 °C) of vegetative shoots and tap roots.

Statistical analyses were performed with the SAS statistical package (Sas Institute Inc. 1985). To compare the means per cohort of several parameters Bonferroni *t*-tests were used after an analysis of variance with the General Linear Models procedure for unequal cell sizes. Pearson rank correlation tests were used on the data on stem length and seed output per plants of all individual plants of both species of *Rumex*.

Photoperiod and flowering of Rumex species

To determine the effects of photoperiod on flowering, plants of both *Rumex* spp. were grown in either 13-h (short day = SD) or 18-h (long day = LD) photoperiods in similar growth rooms (PAR 120 µmol m^{-2} s^{-1}; 400-W sodium lamps, supplemented with 85-W Sylvania GRO-LUX lamps; 15:25 °C [dark:light]).

Starting February 1989, eight series (twelve plants per series per species) were subjected to either 2, 4, 6, or 8 weeks SD or LD and thereafter changed to the other regime. Two additional series remained in either LD or SD to serve as controls. Plant growth was measured in the same way as indicated for the outdoor experiments.

Field observations on Rumex species

This study was done to determine whether flowering in both *Rumex* species is related to the time of germination under field conditions as well as in outdoor plots. Four longitudinal plots (each 10 m x 50 cm) were set out in August 1988 in the Kekerdomse Waard near Nijmegen. The plots were situated parallel to the river at different elevational levels with different flooding frequencies and durations. One of the plots was located in a clay pit at approximately 9.70 m

with respect to mean sea level (Amsterdam Ordnance Datum = NAP). In 1988, this plot was free of flood water from 20 June onwards. Three other plots were placed at different elevational levels (9.25 m NAP, 9.05 m NAP and 8.90 m NAP) on the shore of an old river bed, where the winter-spring floods started to subside on 24 June, 28 June and 22 July, respectively. Within-plot elevational differences ranged from 10 cm in the highest plot (clay pit) to 4 cm in the lowest. As a result of these variations, more than 50% of the intermediate plot on the old river-bed shore remained waterlogged until 18 July. Plant locations were recorded by two coordinates and each plant was followed until November 1988. Once a month the following data were recorded for each plant: survival, number of living leaves, length of longest leaf, phase of development, and stem length.

Results

Phenology in outdoor experimental plots

Chenopodium rubrum

Several developmental features were constant regardless of cohort (Fig. 1). Seedlings possessed an opposite leaf arrangement until the seventh primary leaf became visible. Leaves ten to forty, if present on the main shoot, all attained maximum lengths of 10-16 cm. This value was rather constant per plant, but different between plants from the same or different cohorts. Secondary shoots appeared in the first two leaf axils as soon as the fifth and sixth leaves were observed. In most cases, additional shoots emerged later from the cotyledonary axils. The primary stem remained short during the vegetative stage. In contrast, the first axillary shoots (in leaf axils one-six) grew large, resulting in a rosette-like appearance (Fig. 1).

After transition to the generative phase, small red flower buds appeared on the main stem. These were soon followed by flower buds on the large branches. They developed from the top downwards (see Lang 1965). Flowering was accompanied by an acceleration in leaf initiation and primary stem growth. This closely resembled bolting in rosette species of *Rumex*. Leaf form and degree of branching of the inflorescence were rather variable in *C. rubrum* (see also Cumming 1969; Williams 1969).

In contrast with the characters described above, the timing of flowering and the investment in seed production were strongly affected by the timing of germination. Plants of *C. rubrum* grown in April and May showed vigorous vegetative growth before flowering in long days (> 15 h). The number of primary leaves initiated before flowering was proportional to the temperature sum up to that time (Table 1). This relationship is surprisingly strong considering that durations that were presented as 50% values are rather crude and that each normal summer day corresponds to 6-10 degreedays.

Fig. 1. (Next page) Vegetative development in outdoor experimental plots (plants from cohort II of 1987). *Chenopodium rubrum* at the age of: (a) 2 weeks, (b) 3 weeks, and (c) 5 weeks; *Rumex maritimus* at the age of: (d) 2 weeks, (e) 3 weeks, (f) 4 weeks, and (g) 6 weeks (transition to flowering: early bolting stage); *Rumex palustris* at the age of: (h) 2 weeks, (i) 3 weeks, and (j) 4 weeks. Main leaves are numbered in the order of their appearance; †, the axillary shoot of dead main leaves.

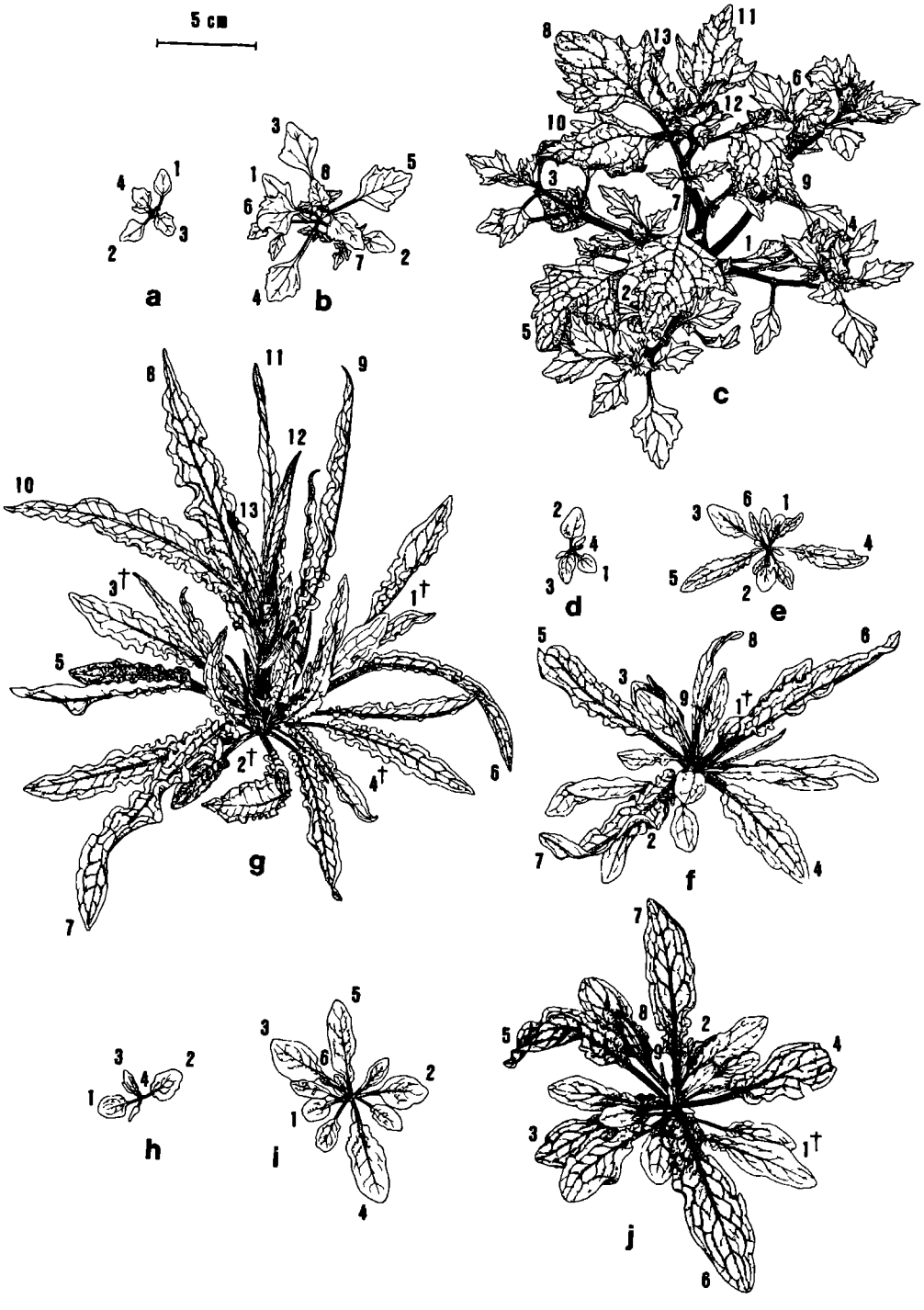
5 cm
a
b
c
d
e
f
g
h
i
j

Table 1. Reproductive development of cohorts of *Chenopodium rubrum*, *Rumex maritimus*, and *Rumex palustris* in outdoor experimental plots.

Cohort	Period of vegetative growth *a*	Weather *b*		% Flowering	Duration *c*			Leaf no. *d*	
		T	R		Veg.	Fl.	Rip.	Veg.	Tot.
C. rubrum									
1987									
I	18 May - 7 Aug.	475	120	100	81	38	24	35	62
II	29 June - 18 Aug.	384	77	100	50	34	28	28	46
III	10 Aug. - 9 Sept.	248	35	100	30	26	41	15	24
IV	21 Sept. - 28 Oct.	105	27	50	37	**	-	0	0
1989									
I	10 Apr. - 25 June	406	127	100	76	50	24	32	82
II	17 Apr. - 25 June	394	120	100	69	50	20	33	78
III	24 Apr. - 30 June	439	131	100	67	48	14	37	80
IV	1 May - 2 July	441	126	100	62	50	12	36	85
V	8 May - 5 July	439	115	100	58	50	14	35	77
VI	15 May - 18 July	537	128	100	64	37	18	40	69
VII	22 May - 27 July	550	128	100	66	32	21	42	68
R. maritimus									
1987									
I	18 May - 7 July	268	78	100	51	37	39	12	32
II	29 June - 9 Aug.	320	66	100	42	40	46	12	36
III	10 Aug.*	-	-	0	-	-	-	-	-
IV	21 Sept.*	-	-	0	-	-	-	-	-
1988									
I	2 May - 15 June	263	75	100	45	37	39	12	33
II	16 May - 28 June	258	66	100	44	35	41	12	31
III	30 May - 11 July	277	62	100	43	37	43	12	32
IV	13 June - 23 July	295	54	100	41	38	43	12	35
V	27 June - 11 Aug.	368	65	25	46	40	46	13	40
VI	11 July*	-	-	0	-	-	-	-	-
1989									
I	10 Apr. - 8 June	241	102	100	60	32	28	13	nd
II	17 Apr. - 10 June	241	98	100	55	30	32	13	nd
III	24 Apr. - 12 June	259	96	100	50	32	34	13	nd
IV	1 May - 12 June	252	86	100	43	32	34	12	nd
V	8 May - 16 June	266	82	100	40	33	36	12	nd
VI	15 May - 23 June	321	85	100	40	35	42	12	nd
VII	22 May - 30 June	310	82	100	40	36	40	12	nd

R. palustris									
1987									
I	18 May - 10 July	289	83	95	54	40	53	12	37
II	29 June*	-	-	0	-	-	-	-	-
III	10 Aug.*	-	-	0	-	-	-	-	-
IV	21 Sept.*	-	-	0	-	-	-	-	-
1988									
I	2 May - 19 June	288	81	100	49	36	49	12	38
II	16 May - 2 July	291	71	100	48	35	50	11	38
III	30 May - 14 July	294	65	100	46	40	51	13	40
IV	13 June - 27 July	326	60	100	45	42	57	13	45
V	27 June*	-	-	0	-	-	-	-	-
VI	11 July*	-	-	0	-	-	-	-	-
1989									
I	10 April - 15 June	307	117	92	67	39	42	14	nd
II	17 April - 15 June	296	111	100	60	42	32	14	nd
III	24 April - 15 June	295	103	100	53	32	33	13	nd
IV	1 May - 16 June	300	96	100	47	32	39	12	nd
V	8 May - 19 June	301	90	100	43	36	37	11	nd
VI	15 May - 25 June	341	90	100	42	36	45	12	nd
VII	22 May - 5 July	356	91	100	45	40	51	13	nd

Note: There were 20 plants per species per cohort in 1987 and 12 in each of 1988 and 1989; nd, not determined. *a* From the time of germination until 50% of the plants had flower buds (*C. rubrum*) or bolted (*Rumex* spp.); *b* Temperature sum (T) in degree-days (10 °C base temp.) and radiation sum (R) as 10^3 Jcm^{-2} during the period of vegetative growth; c Duration (days) of the periods of vegetative growth (Veg.), flowering (Fl., the time until 50% of the plants were out of flower), and ripening of fruits (Rip., the time until 50% of the plants were ripe); *d* Veg., the number of primary leaves (> 1 cm in length) initiated during the period of vegetative growth; Tot., total number of leaves (median values per cohort); *Plants remained vegetative in the year of germination; **Plants died in December (see text).

Plants which started growth after May showed transition to the generative phase correspondingly later in time, but in progressively earlier stages of growth. The duration of flowering correspondingly decreased, as well as leaf increment during flowering. Mean leaf numbers at the onset of bolting as well as mean total leaf numbers were highly significantly different between cohorts I, II and III of 1987. The duration of the period of seed ripening apparently increased, but many of the immature seeds which fell from the latest flowering plants, were readily capable of germination. Smaller plants produced fewer seeds than larger ones, but, plants of the latest flowering cohort allocated up to five times more dry matter into seeds per unit above ground (stem) weight than those of the early flowering cohorts (Table 2). The ratio between the weights of stems and tap roots was constant regardless of cohort (*c.* 3.8 for cohorts I-III, 1987). Mean individual seed weight increased from 0.03 mg for the earliest flowering cohort of 1989 up to 0.09 mg for cohort III of 1987, but mean seed number decreased

from over 200 000 to 2000 per plant for these two cohorts, respectively. Seeds of all size categories required fluctuating temperatures for germination; however, the largest seeds showed a diminished requirement for light (70% germination in the dark compared with 5% in small seeds).

All plants from cohort IV of 1987 died in December after a short period of frost. These plants had six or less primary leaves, all of which were smaller than 1 cm in length. Where seeds had been germinated 2 weeks earlier, the resulting plants produced only a few, large seeds in November (A.J.M. van der Sman, unpublished).

Table 2. Plant size, seed output (mean±1SE, n=20[1987], n=12[1989]) and ratio between seed weight and stem weight (n=10[1987], n=5[1989]) in *Chenopodium rubrum* in outdoor experimental plots.

Cohort	Date planted	Plant height (cm)	Seed weight per plant (g)	Seed wt. to stem wt.
1987				
I	18 May	70.4±1.2*c*	8.6±0.8*bc*	0.89±0.15*b*
II	29 June	53.4±1.6*d*	5.9±0.5*c*	1.28±0.20*b*
III	10 August	21.8±0.6*e*	4.6±0.3*c*	3.87±0.44*a*
1989				
I	10 April	85.0±3.4*ab*	14.5±1.9*ab*	0.79±0.07*b*
II	17 April	81.7±6.1*abc*	13.3±2.5*ab*	0.63±0.11*b*
III	24 April	88.7±2.6*ab*	17.8±1.6*a*	0.62±0.03*b*
IV	1 May	94.0±2.0*a*	16.0±1.0*a*	0.72±0.04*b*
V	8 May	85.9±2.7*ab*	18.6±0.6*a*	0.73±0.02*b*
VI	15 May	78.3±3.9*bc*	14.3±2.1*ab*	0.86±0.03*b*
VII	22 May	76.3±2.4*bc*	15.6±1.0*a*	0.85±0.02*b*

Means followed by the same letter are not significantly different (Bonferroni t-test, p<0.05).

Rumex species

Seedlings of *R. maritimus* and *R. palustris* developed in a similar manner (Fig. 1). Primary leaves emerged successively from a leaf sheath that became fragmented and desintegrated after rainfall. The first five leaves could be recognized easily afterwards by their specific growth habit. At first, seedlings of *R. palustris*, which had originated from larger seeds, were slightly larger than those of *R. maritimus*, but the situation was reversed after the seven-leaf stage. Leaves seven to twelve of both species attained a maximum length of 20-25 cm. As in *C. rubrum*, variation occurred between individual plants from the same cohort and from different cohorts. *Rumex maritimus* possessed relatively longer and narrower leaves than *R. palustris* (Fig. 1), but these differences were not statistically significant. In both species, axillary shoots developed three nodes below newly emerging leaves. The maximum photosynthetic leaf area was usually reached after the twelve-leaf stage. At this stage, transition to the reproductive phase became apparent with the bolting of the main shoot (Fig. 1g). Bolting was accompanied

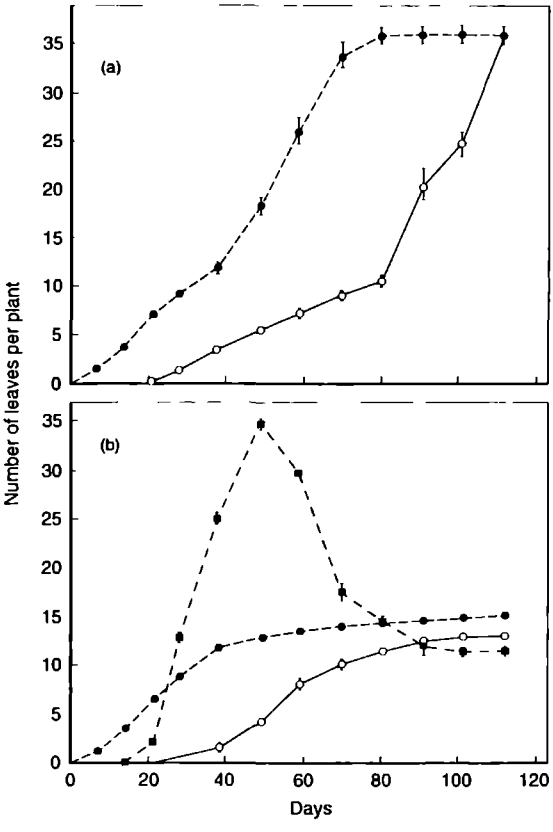

Fig 2. Pattern of leaf initiation and death for two outdoor cohorts of *Rumex maritimus* with the same growth rates up to maximum rosette size (reached after 42 days). (a) The flowering cohort II of 1987; (b) The non-flowering cohort III of 1987. ●, total number of primary leaves; ○, dead primary leaves; ■, number of living axillary leaves (not indicated in (a); see text). Means±1SE (n=20; if not indicated, SE falls within the span of the symbol).

by an increase in the initiation of leaves which is shown for main shoots in Fig. 2a. Flowers appeared approximately 21 days after the onset of bolting. Flowers developed on the upper part of the stem, and also downwards on older stem nodes.

Plants which remained vegetative deteriorated from the time that rosette size was maximum onwards (Fig. 2b). Leaf birth rate decreased, while death rate increased, until after several weeks a constant number of vital leaves remained (*c.* two main and eleven axillary leaves). Apart from this phenomenon, later developing leaves were progressively shorter in length.

In sharp constrast with that in *C. rubrum*, plants of both *Rumex* species flowered at the same phenological stage regardless of treatment, *i.e.* at the time of maximum leaf area (twelve primary leaves). This was reached after a relatively constant temperature sum (Table 1). All cohorts of *R. maritimus* which reached this stage before 11 August (photoperiod 15 h) and those

of *R. palustris* which did so before 27 July (photoperiod 16 h), bolted and flowered. Differences in growth parameters of the main flowering shoot were small between cohorts in drained plants of *Rumex* (see Van der Sman *et al.* 1991, Chapter 7 in this thesis). Mean main stem length in plants of *R. maritimus* was 50 cm, and those of *R. palustris* reached 74 cm. Only plants from the latest flowering cohorts, which flowered with only one main stem, had in some cases significantly shorter stems (*e.g.* the flowering plants of *R. maritimus* from cohort V of 1988). Large differences occurred, however, with respect to the number and size of secondary stems, which elongated from rosette leaf axils in the order of axil appearance. Later flowering cohorts produced fewer, shorter secondary stems, and consequently fewer seeds than earlier cohorts. Total seed weight per plant was highly correlated with total stem length (summed heights of primary and secondary stems) throughout all cohorts (Fig. 3). This was in sharp contrast to that found in *C. rubrum* and indicated a constant mean seed weight to above ground weight ratio.

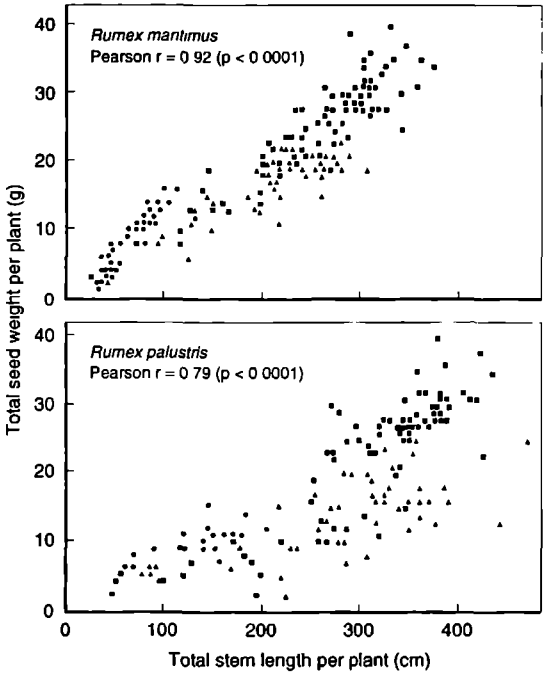

Fig. 3. Total seed production in relation to total stem length (summed heights of primary and secondary stems) in all reproductive plants of *Rumex maritimus* (n=175) and of *Rumex palustris* (n=149) in the outdoor experimental plots.
●, 1987; ▲, 1988; ■, 1989.

Mean individual seed weight per cohort of *R. maritimus* varied between 0.18 mg and 0.27 mg and that for *R. palustris* varied between 0.50 mg and 0.60 mg. The lowest weights were found for cohorts I-III of 1989, which also produced the largest total seed weights and seed numbers of approximately 80 000 (*R. maritimus*) and 30 000 (*R. palustris*) per plant. Statistical analysis of the limited data on individual seed weight was not attempted. No differences in germination

characteristics were found between small and large seeds of both species, but large seeds showed a higher percentage of emergence from up to a 2.5 cm sowing depth (data not shown).

Cohorts emerging over the course of the season showed progressively slower vegetative development, indicated by an increased temperature sum before reaching the twelve-leaf stage, and a slower reproductive development, especially ripening of fruits (Table 1). Within-cohort variation increased as well, and several plants of *R. palustris* from the last cohorts to flower were unable to produce ripe seeds on all secondary stems. Generative development was also delayed in cohorts I and II of 1989 for plants of *R. palustris* which had experienced low temperatures during early vegetative development (Table 1). Occasionally, one plant per cohort of *R. palustris* bolted (*c.* 10 cm) without flowering. This occurred in both flowering and non-flowering cohorts.

Most plants from the later flowering cohorts survived after flowering with vegetative axillary shoots in the rosette leaves. By the end of December 1987, mean dry weights of tap roots from flowering plants ranged from less than 1 g for plants without vegetative shoots remaining to 3 g (*R. maritimus*) and 5 g (*R. palustris*) for plants with three vegetative axillary shoots. By this time, vegetative plants possessed tap roots up to 5 g (*R. maritimus*, cohort III) and 7 g (*R. palustris*, cohort II). Plants from cohort IV of 1987 were unable to reach the maximum rosette size. In November, after 120 degree-days, plants from this cohort possessed up to five main leaves with a maximum length of 3-4 cm, and in December dry weights of tap roots were only 10-15 mg for *R. maritimus* and 30 mg for *R. palustris*. However, most of these very small plants survived 4 months submergence during the winter (see Chapter 6). In the following growing season these small plants flowered and set seed, just as all the other vegetative plants and the remaining vegetative shoots of flowering plants.

Photoperiod and flowering of Rumex species

Plants of *R. palustris* did not flower in this study. Plants of *R. maritimus* all flowered if they recieved 6 or more weeks LD (Table 3), irrespective of SD given afterwards. The main shoot bolted after ten main leaves had been formed. At this stage plants possessed five axillary shoots with a total of eleven axillary leaves. The series that had been placed in SD after 4 weeks LD, resulted in six plants which flowered normally, three of which remained vegetative, and three of which exhibited abnormal growth. The latter started to bolt at the same time as the normally developing plants, but flowering was delayed by a month. A short flowering stem (8.5 cm) was formed, with many small leaves and fewer flowers than other plants. Eventually seeds (0.34 g) were produced, but ripening was delayed and incomplete.

Plants which were grown in various SD periods and then placed in LD subsequently showed a progressive delay in bolting and flowering (Table 3). In all series, however, bolting occurred after approximately ten leaves had been initiated in LD. Duration of flowering increased with the time spent in SD before LD, as did total leaf number, plants height, and seed weight per plant, with the exception of series 8SD/LD (Table 3). This last series produced a relatively low total seed weight and a low ratio between seed weight and above ground (stem) weight. This ratio increased with the period of SD before or after LD in all series up to series 6SD/LD and 8SD/LD. By the time these series had flowered, plants had grown for over three quarters of a year on the limited substrate in the small pots and a shortage of nutrients may well have occurred.

Table 3. Reproductive development of plants of *Rumex maritimus* in different photoperiodical regimes. Plant height, total seed weight, and ratio of seed weight to stem weight are presented as mean (±1SE, n=12) per plant.

Series *a*	Flowering (%)	Duration *b*			Leaf no. *c*			Plant height (cm)	Seed wt. per plant (g)	Seed wt. to stem wt.
		Veg.	Fl.	Rip.	Veg.	LD	Tot			
SD	0	-	-	-	-	-	-	-	-	-
2LD/SD	0	-	-	-	-	-	-	-	-	-
4LD/SD	50*	34	43	21	10	7	25	27.7±1.8*c*	0.96±0.09*bcd*	2.23±0.15*ab*
6LD/SD	100	34	43	21	10	10	24	27.5±1.1*c*	0.76±0.05*d*	1.89±0.13*abc*
8LD/SD	100	34	43	21	10	10	23	25.9±0.8*c*	0.66±0.05*d*	1.68±0.04*bc*
LD	100	34	40	24	10	10	24	23.8±0.8*c*	0.82±0.05*cd*	1.74±0.09*bc*
2SD/LD	100	48	39	24	12	11	27	24.8±0.5*c*	1.19±0.10*abc*	2.26±0.17*ab*
4SD/LD	100	72	47	21	15	10	34	24.6±1.2*c*	1.27±0.12*ab*	2.58±0.24*a*
6SD/LD	100	111	68	24	19	10	42	35.3±2.0*b*	1.40±0.10*a*	2.02±0.20*abc*
8SD/LD	100	133	58	16	21	12	43	42.1±1.8*a*	1.08±0.11*abcd*	1.34±0.09*c*

a SD (short day) = 13 h, LD (long day) = 18 h, 2LD/SD = 2 weeks LD and SD thereafter, etc.; *b* Duration (days) of the periods of vegetative growth (Veg., until 50% bolting), flowering (Fl.), and seed ripening (Rip., 50% values; see Table 1); *c* Veg., number of primary leaves (> 1 cm in length) initiated during the period of vegetative growth, LD, number of leaves formed in LD, Tot., total number of leaves (median values per series); *Another 25% flowered but exhibited abnormal growth (see text) and is therefore excluded. Means followed by the same letter are not significantly different (Bonferroni t-test, p<0.05).

Field observations on Rumex species

The largest plants of both *Rumex* species were found in the highest of the three plots on the river-bed shore (approximately five leaves per plant in August, length of longest leaf 20 cm), followed by the plot in the clay pit (four leaves, leaf length 7 cm). Within-plot variation was much larger in the clay pit than on the relatively flat river-bed shore The two lower plots on the river-bed shore did not differ significantly from each other with respect to plant size (three leaves, leaf length 3 cm). In the lowest plot, newly germinated seedlings were found during the second observation in September.

Flowering occurred only in plants growing in sites where the flood-water had subsided before the end of June. Flowering of plants of *R. maritimus* in the intermediate plot on the river-bed shore occurred only in the higher parts. In all plots more large plants flowered than small ones (data not shown). A significantly higher percentage of plants of *R. maritimus* flowered compared with those of *R. palustris* (Table 4). All flowering plants had already bolted on 10 August, with the exception of five plants of *R. maritimus* from the clay pit. In October, seeds on all generative plants of *R. palustris* and several plants of *R. maritimus* in the clay pit were ripening. The remaining plants of *R. maritimus* were in full flower. The last field observations in the other plots were made in September because the river bed was flooded for several weeks in October and this resulted in the death of all reproductive plants. At that time most plants of *R. maritimus* had just flowered and those of *R. palustris* were still in bud. All flowering plants had only one main stem. Only those of *R. maritimus* from the highest plot on the river-bed shore

attained a mean stem length which was comparable to the plants in the outdoor plots (46 cm). Plants in the clay pit remained much smaller than in the outdoor plots (*R. maritimus* 18 cm; *R. palustris* 33 cm). More than 80 % of the flowering plants in this plot survived until seed ripening. Loss of plants was primarily caused by predation of the chrysomelid beetle *Gastrophysa viridula.*

Table 4. Percent flowering plants (*n* between brackets) in September 1988 of *Rumex maritimus* and *Rumex palustris* in four plots (each 10 m x 50 cm) at different elevations in two sites of the river forelands near Nijmegen, The Netherlands.

Site	Plot	NAP*	Start of the season**	*R. maritimus*	*R. palustris*
Clay pit	1	9.70	20 June	63(30)	17(63)
River bed	2	9.25	24 June	58(36)	25(16)
River bed	3	9.05	28 June - 18 July	6(31)	0(75)
River bed	4	8.90	22 July	0(15)	0(28)

*Mean height (m) above sea level (Amsterdam Ordnance Datum); **Subsidence of winter-spring floods. Differences in percent flowering between species are highly significant. Differences between plots 1 and 2 are not significant. Differences between plots 1 and 2 on one hand and plot 3 on the other are highly significant for *R. maritimus* (χ^2, $p<0.001$).

Discussion

The controls of flowering and seed production are quite different for *C. rubrum* on one hand and for *R. maritimus* and *R. palustris* on the other. They have contrasting photoperiodic responses. In the short-day plant *C. rubrum,* flowering occurred as a response to daylength and appeared relatively independent of the amount of vegetative growth. In the two long-day species of *Rumex*, stem elongation and subsequent flowering always occurred after a rather constant number of leaves had formed. All three species fit into the life-history classification which Klinkhamer, de Jong & Meelis (1987) suggested for short-lived species based on the control of flowering. In riparian habitats both control types of flowering can result in the production of large numbers of seeds should floods recede early enough in the growing season.

According to Cook (1976), three factors determine potential seed number in *C. rubrum.* These are: (i) the total number of axillary bud primordia on the plant at the start of flower induction, (ii) the increased rate of organ initiation during the process of induction, and (iii) the duration of this higher rate of organ initiation. Cook demonstrated that the last two factors are affected by the induction photoperiod. The early rate of organ initiation was higher in a longer photoperiod than in a shorter one, and the rate of differentiation, which determines the duration of this higher rate of organ initiation, was lower. Our findings agree with those of Cook, for example, in outdoor plots both the duration of flowering and leaf increment during flowering decreased in late-induced plants of *C. rubrum* (Table 1). Under natural conditions the first factor is also related to photoperiod; long days favour vegetative growth in *C. rubrum* (Cumming 1961). Our results show that large, early germinated plants also started to flower earlier than late germinated plants, in a longer photoperiod. Thus, photoperiod clearly determines the potential

seed output of this species, resulting in very large differences between plants grown at the extremes of the growing season.

Seed size is determined by seed number which in turn is governed by the photoperiod during flower induction, and natural induction tends to minimize seed size and maximize seed number in *C. rubrum* (Cook 1975). There is probably little need for the competitive advantage of large seedlings which germinate from large seeds in these open habitats. However, a high capacity for germination in the dark together with their size, may give larger seeds a better chance to emerge from deeper soil layers on sandy river beaches or from cracks in the clay of old river beds (see Harper, Lovell & Moore 1970). We not only found larger seeds on plants flowering in short days but also up to a five times larger seed output with respect to other plant parts (stem and tap root) in comparison to plants flowering in long days (Table 2). The latter showed a partioning of biomass comparable to that described by Williams (1969) for this species. This indicated an increasing reproductive effort in plants of *C. rubrum* nearer the physiologically optimum photoperiod for flowering.

Flowering in both species of *Rumex* occurred during and after long days, after a minimum number of leaves had been formed (see Lang 1965; Vince-Prue 1975; Bernier, Kinet & Sachs 1981a). These species are probably sensitive to flower induction shortly after germination, since short days during early development delayed flowering in *R. maritimus* and resulted in an increase in the number of leaves before bolting occurred (Table 3). The development of stems, flowers and seeds was not influenced by short days. Between-cohort differences in the rate of development in outdoor plots were therefore probably mainly caused by temperature. The minimum number of leaves initiated before flowering (especially the number of secondary leaves) is reduced in small pots and under poor nutrient conditions (A.J.M. van der Sman, unpublished). By this means these species maintain approximately the same flowering time under greenhouse conditions, which is advantageous when considering the time required for reproductive development (see Bradshaw 1965). In the field study, plants were smaller and much more variable in the clay pit than on the river bed shore, but reproductive development was not delayed. This may be caused partly by a lower nutrient availability in the clay pit than on the river-bed shore. Another cause may have been the larger elevational differences in the clay pit; at least part of the plants had probably germinated later than 20 June.

The diminished seed output shown by June plants of *Rumex* spp. mainly resulted from the smaller number and size of secondary stems when compared with earlier germinated plants. In the indoor study, secondary shoots of *R. maritimus* remained very small and did not flower. In outdoor plots, the oldest (largest) shoot elongated first. Therefore, bolting and flowering of secondary shoots is probably not determined by the main shoot but simply dependent upon their size (leaf number) with respect to photoperiod (see also Lang 1965).

After a certain critical photoperiod, no flowering of *Rumex* occurred at all. Plants of *R. palustris* shifted to the (partly) vegetative strategy earlier in the season in outdoor experiments and in the field plots fewer plants of *R. palustris* flowered than of *R. maritimus* (Tables 1 and 4). The longer critical photoperiod for flowering in *R. palustris* was related to the time needed for reproductive development, compared with *R. maritimus*, which was longer and also more susceptible to temperature (see also Hejny 1960). Flowering in *R. palustris* was not simply induced by LD in this study or in other experiments under the conditions provided (>16 h photoperiod; dark temperature 17 °C). High temperatures during the dark period inhibit flowering in several long-day species (Lang 1965; Vince-Prue 1975). This factor may be crucial for *R. palustris*, illustrated by the observation of 100% flowering in an unheated greenhouse

with a night temperature of 10 °C (A.J.M. van der Sman, unpublished). Adult plants have a cold requirement before flowering occurs in the greenhouse in LD. The relationship between induction by LD and vernalization may be extremely complex in species which are sensitive to both (Bernier, Kinet & Sachs 1981a).

Plants of both *Rumex* spp. which remained vegetative in short days exhibited a marked reduction in above-ground growth from the time of maximum leaf area onwards. This may be important for their cold-hardening, as has been suggested for certain grasses (Hay 1990). This reduction in growth was apparently related to investment in tap roots. We suppose that in the river forelands, with frequently occurring prolonged winter and spring floods, *R. palustris*, and to a lesser extent *R. maritimus*, will show a tendency towards longer life histories involving more than one growing season. In contrast, the strictly annual *C. rubrum* will only complete its life cycle in a shorter time, if germinated later in the growing season (until as late as September). Therefore, survival of *Rumex* populations will probably depend more upon their relative flooding resistance in the established phase than that of populations of *C. rubrum*. This will be the subject of the following Chapters.

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FLOODING REGIMES AND LIFE-HISTORY CHARACTERISTICS OF SHORT-LIVED SPECIES IN RIVER FORELANDS

with N.N. Joosten and C.W.P.M. Blom

A large-scale outdoor experiment was carried out to study the effect of flooding on growth, survival and reproduction in relation to developmental stage in three short-lived species. Several cohorts of *Chenopodium rubrum*, *Rumex maritimus* and *Rumex palustris* were raised in accordance with a flooding regime that was based on the average flooding conditions of their natural habitats in river forelands.
Survival of submergence in the pre-reproductive phase was high in both species of *Rumex*, but relatively low in *C. rubrum*. Biomass reduction following flooding depended on plant size before flooding and the mean temperature of the flood-water. Both *Rumex* spp. were less reduced in size during flooding, and better capable of regeneration afterwards than *C rubrum*.
Later-raised cohorts of the short-day species *C. rubrum* started to flower after a shorter time and at an earlier developmental stage than earlier cohorts. Flooding had little effect on probability of flowering and caused only a slight delay in flowering in this species. In both long-day species of *Rumex*, flowering stems were not initiated until a minimum leaf number was formed. As a result later cohorts remained vegetative, while flooding delayed flowering until later in the season or even until the following year.
All flooded plants of *C rubrum* and most flooded plants in early cohorts of *R maritimus* attempted to reproduce in the first growing season. Seed number was severely reduced in *C rubrum* due to both reduction of plant biomass and destruction of flowers during flooding. In flooded plants of *R maritimus*, seed output was correlated with plant size at the time of bolting. Seed number was maximized at the expense of seed size in *R maritimus*.
These results suggest that there is a trade-off between survival of flooding and early reproduction in these species, and that there are complex inter-relationships between highly variable environmental factors and developmental factors which determine survival and reproductive success of short-lived species in river forelands.

Introduction

Flooded zones along rivers in The Netherlands are bounded by the main dykes at either side of the river. These ribbon-like zones up to several kilometres wide are referred to as forelands. Differences in elevation, both natural and man-made, combined with strongly fluctuating water-levels resulting from unpredictable changes in river-water discharge, lead to highly dynamic hydrological regimes in space and in time in these areas (Van de Steeg 1984; Brock, Van der Velde & Van de Steeg 1987; Blom 1990; Blom *et al.* 1990). The occurrence of several *Rumex* species in restricted zones along elevational gradients in these forelands suggests that these species differ in flooding tolerance. Comparative studies concerning different aspects of flooding tolerance in *Rumex* revealed a dichotomy between species from high elevations on the one hand and species from low and intermediary elevations on the other (*e g.* Laan et al. 1989; Voesenek & Blom 1989b; Voesenek, Blom & Pouwels 1989; Laan & Blom 1990; Laan *et al.* 1990). These studies were mostly performed on young vegetative plants under controlled conditions where flooding was treated as a stress factor (*sensu* Grime 1979). In the lowest areas of river forelands, flooding will be primarily experienced as a disturbance by short-lived ruderal species (Menges & Waller 1983). Floods may occur at several stages of the life cycle and these stages should all be included in studies concerning flooding tolerance. For example, *Rumex*

species from frequently flooded areas have persistent seed-banks, whereas species from higher elevations have transient ones (Voesenek & Blom 1992a). A distinction between avoidance strategies, such as survival through periods of adverse conditions by means of persistent seeds, and tolerance strategies, such as metabolic adaptations, is extremely useful in this respect (Crawley 1986).

In order to study the interaction between natural flooding regimes and reproductive behaviour of established plants, three co-occurring species from low, frequently flooded areas of river forelands were selected. *Chenopodium rubrum* L., *Rumex maritimus* L. and *Rumex palustris* Sm. are short-lived species with different life histories. Both *Rumex* spp. are annual if they reach a mature vegetative size before a certain photoperiod has passed (Van der Sman, Blom & Van de Steeg 1992, Chapter 3 in this thesis). This photoperiod is longer for *R. palustris*, which displays the biennial strategy more often in the river forelands than *R. maritimus*. In contrast to both *Rumex* spp., the short days of late summer and autumn promote early flowering in the strictly annual *C. rubrum*. In a greenhouse experiment, *R. maritimus* appeared to be much more tolerant towards flooding than *C. rubrum* (Van der Sman, Van Tongeren & Blom 1988, Chapter 2 in this thesis), but duration, time and frequency of flooding were all factors which considerably modified growth, the onset of flowering and seed production within one or both species. Plant developmental stage has been shown to play an important role in the rapid under-water elongation response of shoots in *R. maritimus* (Van der Sman *et al.* 1991, Chapter 7 in this thesis).

A large-scale outdoor experiment was designed to enable the simultaneous comparison during one growing season of several realistic flooding conditions. Several cohorts of plants of *C. rubrum*, *R. maritimus* and *R. palustris* were raised in accordance with these flooding regimes to study the effect of flooding on growth, survival and reproduction in relation to plant developmental stage. Species differences in flooding tolerance were discussed in relation to differences in life-history characteristics.

Materials and methods

Habitat description and plant material

Chenopodium rubrum is one of the character-species of the *Chenopodietum glauco-rubri* Lohm. and both *R. maritimus* and *R. palustris* are character-species of the *Ranunculo-Rumicetum maritimi* Siss. (Westhoff & Den Held 1975). *C. rubrum* occurs as companion species in the *Ranunculo-Rumicetum* association and, likewise, both *Rumex* spp. occur in the *Chenopodietum*. In the river forelands several plant communities exist which may be interpreted as incomplete stages of these associations or as transitional stages between them (H.M. van de Steeg, unpublished relevés). The *Chenopodietum* may occur on exposed clay soils as well as sandy soils, especially flood-marks on sandy river beaches, while the *Rumicetum* is more characteristic of wet clay soils. Both *Rumex* spp. also occur in associations of marsh species (*Oenanthion*, Westhoff & Den Held 1975). Germination of plants of *C. rubrum* and both *Rumex* spp. occurs after the subsidence of floods from April onwards and multiple post-flooding cohorts are commonly observed as described for *R. palustris* and *R. crispus* in Voesenek & Blom (1992a). Maximum germination in the laboratory is achieved under conditions of light and fluctuating

temperatures in all three species. Such patterns appear to be common in wetland species (Thompson & Grime 1983; see also Voesenek & Blom 1992b).

The flooding regime to be used in the outdoor experiment was derived from water-level measurements which were made in the period 1970-87 and analysed in relation to the elevation of three study areas near Nijmegen, The Netherlands (see also Chapter 1). These sites were a clay-pit, a sandy river beach and a former river bed. These sites provide examples of the habitats in which the above-described plant communities occur in river forelands. The clay-pit area was partly protected by a summer dyke, which contains a sluice that is closed after the subsidence of winter-spring floods to prevent further flooding during the growing season. In unprotected areas, flooding frequency was highly variable between years but in years and sites in which winter floods subsided early (April-May), the mean number of floods was three to four per growing season. Mean duration ranged from 10 days of flooding in the highest sites (clay-pit area) to 16 in the lowest (river-bed area). Water depth for the majority of floods was less than 1 m above the soil surface. In the river-bed area, extremely severe floods, *i.e.* those with a duration of more than a month and maximum water levels of several metres above the soil, occurred approximately once every three years. Such floods are lethal to established plants in the growing season (Voesenek 1990) and for this reason a moderate regime of four 10-day flooding periods with a constant depth of 40 cm was chosen for the experiment.

Seeds of *C. rubrum*, *R. maritimus* and *R. palustris* were harvested in 1986 in the clay-pit area inside the summer dyke. The total seed output from five plants per species was mixed and dry-stored in the laboratory.

Outdoor flooding experiment

Uniform germination was achieved as described previously (*e.g.* Chapter 3). After 4 days, germinating seeds were transplanted into pots (diameter 16 cm, height 50 cm) filled with a 1:1 mixture (v:v) of river sand and peat. Seven seedlings were transplanted per pot and thinned to one seedling per pot after 2 weeks. Four cohorts each of fifty pots per species were raised in large basins of water (length x width x depth: 8 x 2.4 x 1 m). Four basins, two of which were flooded, were divided lengthwise and each cohort was split between two basin halves with parallel arrangements in the one to be drained and the one to be flooded. The first cohort was sown on 14 May 1987, 32 days before the start of the first 10-day flooding period (Fig. 1). The following cohorts were sown at 6-week intervals, immediately after subsequent flooding periods, on 25 June, 6 August and 17 September, respectively. Drained conditions were provided by a water level of 5-10 cm in the basins. To simulate flooding according to the regime shown in Fig. 1, basins were filled with tap-water until the water level reached an overflow, approximately 40 cm above the soil level in the pots. At this point the water depth in the basins was 90 cm. After 10 days, the excess water was pumped out. Filling and emptying the basins both took approximately 8 h.

Measurements

Irradiation and temperature were continuously monitored during the study period. These parameters are presented for each cohort as radiation and temperature sums (with 10 °C as base temperature, Chapter 3) over the growth period up to first flooding. During flooding periods, water temperatures were measured twice daily, at approximately 08.00 h and 17.00 h. Before and after each flooding period (Fig. 1) the following parameters were measured for all plants: the cumulative number of leaves > 1 cm in length on the main shoot (Chapter 3), the number of

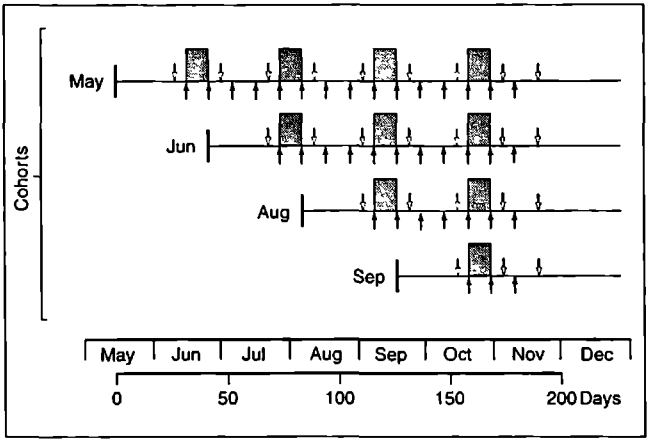


Fig. 1: Experimental design of the outdoor flooding experiment. Growth periods of four cohorts of *Chenopodium rubrum*, *Rumex maritimus* and *Rumex palustris* in relation to four 10-day flooding periods (small shaded blocks). Per cohort and species, 20 plants were kept drained and 20 were flooded. Alternate months are indicated with a light tone. Measurement times: ↓all plants, ↑five plants per species and treatment.

dead main leaves, the number of axillary shoots, the length, width and petiole length of the largest leaf, and the length of the main stem. Before and after the first flooding period of each cohort, three plants per species and treatment were harvested to determine the dry weight of shoots, the maximum diameter (r) of tap roots, and the length (h), if any, of this root which was > 1 mm in diameter. The volumes of tap roots were estimated from these parameters as $0.5\pi r^2 h$. Of the remaining twenty plants per species, cohort and treatment, five randomly selected plants were measured every 10 or 11 days (Fig. 1) with respect to the above-mentioned non-destructive parameters, as well as the number of living axillary leaves (> 1 cm) and the lengths of axillary stems. Twice a week, the developmental stage of all plants was recorded, distinguishing the following stages: vegetative, bolting (*Rumex* spp.), flower buds visible, flowering, ripe fruits (*R. maritimus*: golden brown; *R. palustris*: dark red brown; *C. rubrum*: black). Ripe seeds (including perianths) were collected in November and weighed after sieving and cleaning several times. This was done for all reproductive plants of both *Rumex* spp. and for a maximum of ten plants per cohort and treatment of *C. rubrum*. For each species, cohort and treatment, five samples of 100 seeds were taken from each of three plants. After removal of perianths, seeds were counted and weighed to determine mean seed weights. The empty perianths were also weighed and from the mean values of these fifteen samples per species, cohort and treatment, the total number of seeds was estimated for all plants.

Germination characteristics

Seeds were incubated under the following conditions: constant temperatures of 10 °C and 20 °C, and fluctuating temperatures of 12 h at 20 °C and 12 h at 10 °C, both in the light (15 $\mu molm^{-2}s^{-1}$ PAR during the 12-h day) and in the dark. Three samples of fifty seeds each from a mixture of five plants per species, cohort and treatment were incubated in each of the above regimes in petri-dishes on filter paper (Schleicher & Schüll 595) watered with demineralized water. As soon as radicles appeared, germinated seeds were removed. After 3 weeks, ungerminated seeds were placed into the regime with fluctuating temperatures and light, which normally induced near 100% germination (Chapter 3). Ungerminated seeds were stained with tetrazolium chloride (1 % weight/vol., Sigma) which colours live tissue red (Moore 1972).

Data analysis

All tests were carried out with the SAS statistical package (SAS Institute Inc. 1985). Correlation analysis was performed using the Pearson rank correlation test. The General Linear Models procedure was used for all unbalanced analyses of variance. Significance of differences between means were tested with a Student's *t*-test. In *C. rubrum* and *R. maritimus*, between-plant differences in mean seed size were tested as a nested effect within the effects of cohort and flooding treatment. In the former species, the effects of cohort and flooding treatment were not tested separately but as one main treatment effect because too many cells were empty in the case of flooded plants, due to plant death.

Results

Flooding tolerance in the pre-reproductive phase

Plant biomass is correlated with the product of the number of living leaves and maximum leaf length in these species (Chapter 2; Voesenek 1990). Therefore these parameters were chosen to present plant growth in relation to radiation and temperature sums up to the first flooding period per cohort (Table 1). Growth rates appeared to depend on temperature during the larger part of the growing season (see also Chapter 3). Radiation was probably only limititing in the last, September cohort (Table 1).

The initiation of leaves stopped during the period of first flooding for all cohorts while leaf senescence on the main shoot was high only in the first three cohorts (Fig. 2). In addition, most axillary shoots died. In both *Rumex* species, the oldest leaves of the main shoot died first. In *C. rubrum*, old as well as young leaves died. In the June and August cohorts, mean above-ground dry weight was reduced immediately after the first flooding period by 90% in *R. palustris*, 95% in *R. maritimus* and 98% in *C. rubrum*, compared with drained plants of the same age. In the May cohort, above-ground biomass of both *Rumex* spp. was reduced by as much as 99% and *C. rubrum* was killed by flooding. In the September cohort, plants were much less affected in size by flooding and survival was high in all three species (Table 1). Tap roots were only large enough to be measured after flooding in the second and third cohorts; mean estimated volumes were reduced by 86% in *R. palustris*, 91% in *R. maritimus*, and 93% in *C. rubrum* in these cohorts. In accordance with these results, *C. rubrum* showed the highest mortality in all cohorts (Table 1).

Vegetative growth in relation to the flooding regime

During submergence periods, the orientation of leaves and branches (*C. rubrum*) changed from horizontal to vertical within a day in all three species. In addition, all vegetative plants of both *Rumex* spp. exhibited fast extension of the petioles of young leaves (Fig. 3). The water surface was not reached by any of the plants. The extended leaves were easily damaged and did not survive for more than a few days or, exceptionally, a few weeks after submergence. After submergence, plants of both *Rumex* spp. resumed normal growth patterns. Plants of *C. rubrum* recovered from submergence by developing new leaves from the apex and/or a few scattered leaf axils but they exhibited a stunted growth habit, quite different to that of drained plants. Plants of all three species which survived the first flooding period also survived following flooding periods.

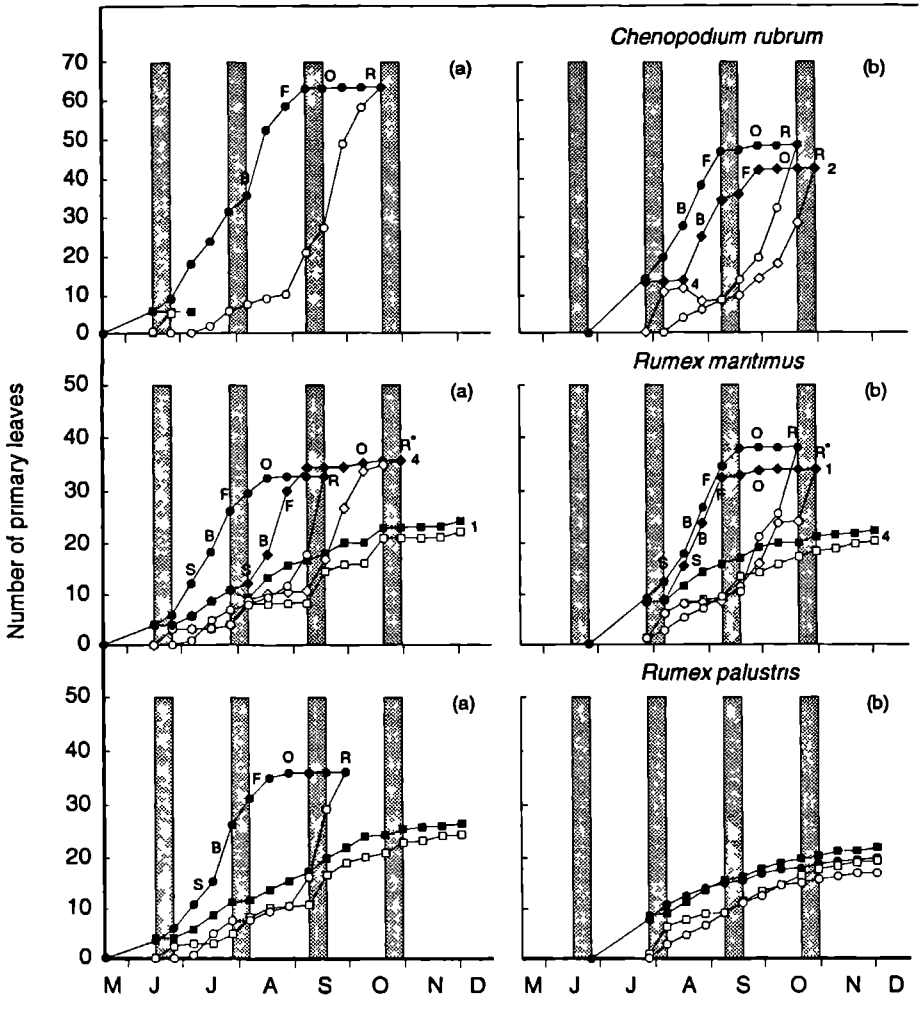


Table 1: Total number of living leaves (>1 cm) and maximum leaf length (mean±1SE, *n*=5) immediately before first submergence in relation to temperature sum (base temp 10 °C) and radiation sum over the first 32 days of growth in *Chenopodium rubrum*, *Rumex maritimus* and *Rumex palustris*. Percentage survival (*n*=20) after the first 10-day flooding period is presented in relation to the mean temperature of the flood water.

Cohort	Temperature sum	Radiation sum	*C rubrum*		*R maritimus*	
	(degree-days)	(Jcm $^{-2}$)	Leaf no	Leaf l. (cm)	Leaf no	Leaf l (cm)
May	103	40 000	6.0±0 0	2.0±0.2	4.0±0.0	2.4±0.1
June	254	52 000	32.8±2 5	9 3±0 6	21 2±1 3	11.6±0 5
August	238	35 000	23 0±2.4	5.2±0.3	21.6±0 8	9 8±0.4
September	92	22 000	0 0±0 0	0 2±0 1	2.0±0 0	1 1±0 1

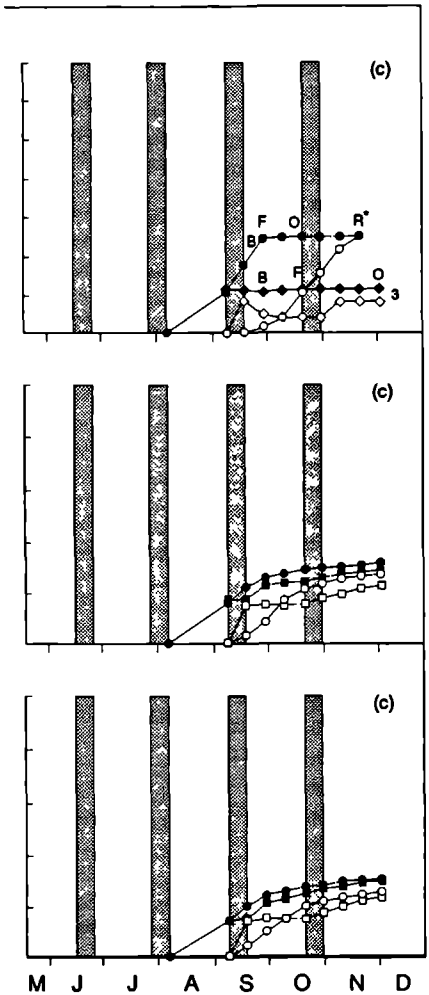
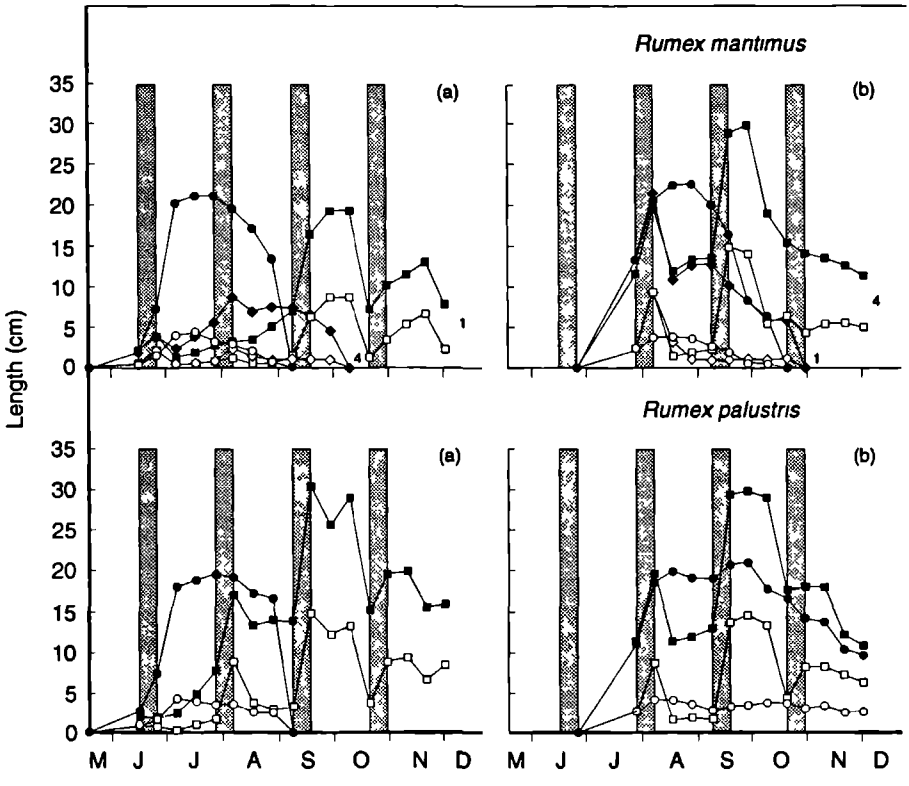


Fig. 2: Cumulative initiation (closed symbols) and loss (open symbols) of primary leaves during the course of the outdoor experiment. Per species and cohort: (a) May, (b) June, (c) August; the September cohort is omitted. Shaded areas indicate flooding periods. ●, drained plants; ■ , vegetative flooded plants; ◆ , reproductive flooded plants (mean, n=5 or indicated if less). Reproductive development of the main shoot is indicated with letters: S=stem elongation (*Rumex* spp.), B=appearance of buds, F=in full flower, O=out of flower, R=ripe (R*=incompletely ripened).

Table 1 continued:

R. palustris		Mean water	Survival (%)		
Leaf no.	Leaf l. (cm)	temperature (°C)	*C. r.*	*R. m.*	*R. p.*
3.8±0.2	2.1±0.2	14.2	0	85	100
23.6±1.2	11.6±0.4	17.1	65	100	100
18.4±0.4	9.0±0.2	17.8	50	100	100
2.0±0.0	1.4±0.2	10.3	95	100	100



Reproductive development in relation to the flooding regime

Transition to the reproductive phase started with rapid stem elongation in the caulescent species *C. rubrum* as well as in the two rosette species of *Rumex* (Fig. 4, see also Bernier, Kinet & Sachs 1981b). All surviving plants in the first three cohorts of *C. rubrum* flowered regardless of treatment (Table 2). Some plants in the September cohort initiated flower buds but they were unable to produce seeds before they were killed by a short period of frost in December. Flowering was not much delayed by flooding in *C. rubrum* (Fig. 2).

Flowering occurred in the May and June cohorts of *R. maritimus* and in the May cohort of *R. palustris* (Table 2). Seed output was correlated with the number and size of axillary stems in drained plants of both species (Table 3). Flooded plants of *R. palustris* all remained vegetative, while flowering percentage was reduced in flooded plants of *R maritimus* (Table 2). Flowering occurred after approximately twelve primary leaves were initiated, in drained as well as flooded plants of *R. maritimus* (see also Chapter 3). At this stage, flooded plants possessed fewer (< 4) axillary leaves than drained plants (*c.* 45), and they did not produce axillary stems. Plants of

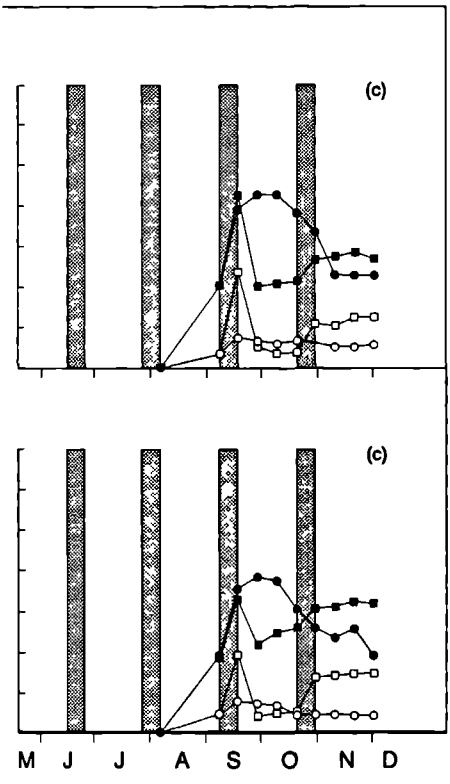


Fig. 3: Maximum leaf lenght (closed symbols) and petiole length (open symbols) in the course of the outdoor experiment in *Rumex maritimus* and *Rumex palustris*. Cohorts: (a) May, (b) June, (c) August; the September cohort is omitted. Shaded areas indicate flooding periods. ● , drained plants; ■ , vegetative flooded plants; ◆ , reproductive flooded plants (mean, n=5 or indicated if less).

both *Rumex* spp. which remained vegetative either reached the twelve-leaf stage too late in the season or did not reach this stage at all before the onset of the winter (September cohort).

Table 2. Percentage flowering (number of plants which survived up to flowering between brackets) per cohort and treatment in *Chenopodium rubrum*, *Rumex maritimus* and *Rumex palustris* (see Fig. 1 for details).

Cohort	*C. rubrum*		*R. maritimus*		*R. palustris*	
	Drained	Flooded	Drained	Flooded	Drained	Flooded
May	100(20)	-(0)	100(20)	88(17)	95(20)	0(20)
June	100(20)	100(13)	100(20)	35(20)	0(20)	0(20)
August	100(20)	100(10)	0(20)	0(20)	0(20)	0(20)
September	45(20)	21(19)	0(20)	0(20)	0(20)	0(20)

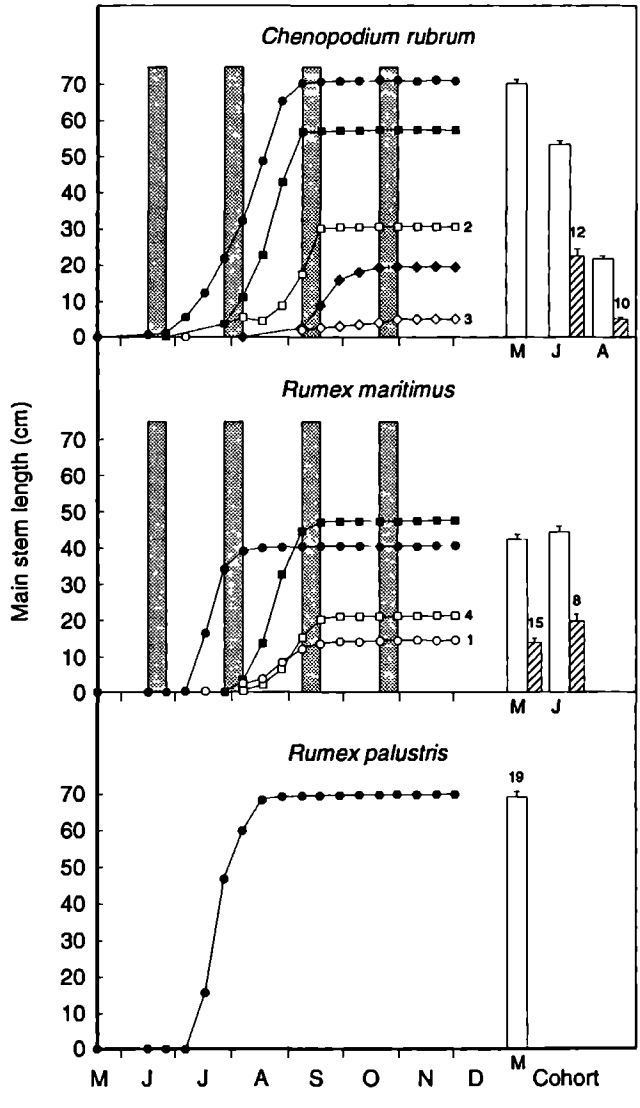


Fig. 4: Mean length of the main stem (n=5 or indicated if less) per species, cohort (●May; ■ June;◆August) and treatment (closed symbols: drained; open symbols: flooded [shaded areas indicate flooding periods). Mean (±1SE, n=20 or indicated if less) final heights of all flowering plants are presented as bars (open: drained; striped: flooded). Cohorts: M= May, J=June, A=August.

Submergence reduced the final size of reproductive plants compared with drained conditions. Flowering plants of *R. maritimus* produced approximately the same total number of main leaves under flooded as under drained conditions (Fig. 2) but stems of flooded plants remained significantly smaller (Fig. 4), while plants of *C. rubrum* were reduced in both respects after flooding. In addition, submergence of flowering plants resulted in severe loss of flowers and only a small number of seeds was produced by flooded plants (Table 4). Only two plants of *C.*

rubrum in the August cohort, which was in full flower just before the start of the last flooding period, produced a few large seeds. In *R. maritimus*, however, new flowering shoots developed from axillary nodes of stem leaves after flooding. This resulted in a relatively constant seed output which was correlated with plant size (biomass estimated from the product of the number of living leaves and maximum leaf length) at the time of bolting (Pearson rank correlation coefficient 0.78, n=22, p<0.001).

In *R. maritimus*, mean seed weight was significantly lower in flooded plants than in drained plants (Table 4). Fruits of flooded plants appeared to be incompletely ripened (Fig. 2) and possessed seeds of a lighter colour than drained plants. Variation in seed weight was largely determined by flooding treatment (73%) and inter-plant differences (20%) in *R. maritimus*; less than 1% of the total sum of squares was attributable to cohort effect. In *C. rubrum*, only half of the flooded plants in the June cohort produced many seeds (Table 4). Mean seed weight was significantly higher in the flooded plants but variation in seed weight was only partly determined by overall treatment effect (51%) and inter-plant differences (2%) in *C. rubrum*.

Table 3. Correlations (Pearson rank correlation coefficient) between stem parameters and total seed weight per plant in plants of *Rumex* spp. grown under drained conditions.

	R. palustris n=19	*R. maritimus* n=40
Axillary stem number	0.73 ***	0.81 ***
Σ Axillary stem length	0.65 **	0.88 ***
Primary stem length	0.39 NS	-0.16 NS
Σ Total stem length	0.70 ***	0.89 ***

Significance levels: NS=not significant; **p<0.01; ***p<0.001.

Under fluctuating temperatures in the light, maximum germination was reduced in seeds from flooded plants (>80%) compared to seeds from drained plants (>98%) in both *C. rubrum* and *R. maritimus*. Ungerminated seeds appeared to be dead. Under all other conditions, seeds from flooded plants showed higher germination percentages than those of drained plants. At constant temperatures in the light, seeds from flooded plants showed 10-30% germination and from drained plants 0-10%. In the dark, seeds from flooded plants showed approximately the same germination percentages as in the light under all temperature conditions, whereas in darkness seeds from drained plants hardly germinated under constant temperatures and showed highly variable, but reduced germination under fluctuating temperatures.

Table 4. Mean (±1SE, *n* as shown) estimated seed number per plant and mean (±1SE, *n*=15) individual seed weight per cohort and treatment in *Chenopodium rubrum*, *Rumex maritimus* and *Rumex palustris*.

Cohort	(*n*/*n*)*	Estimated mean seed number		Mean seed weight (mg)**	
		Drained	Flooded	Drained	Flooded
C. rubrum					
May	(10/0)	53 000±2000*a*	-	0.061±0.003*b*	-
June	(10/6)	44 000±1000*b*	990±200*d*	0.065±0.002*b*	0.085±0.004*a*
Aug.	(10/2)	27 000±3000*c*	<10	0.092±0.004*a*	-
R. maritimus					
May	(20/15)	23 000±2000*a*	850±160*c*	0.217±0.007*a*	0.120±0.005*b*
June	(20/7)	13 000±2000*b*	950±240*c*	0.211±0.007*a*	0.108±0.010*c*
R. palustris					
May	(19/0)	8200±400	-	0.593±0.005	-

*Drained/flooded plants; of the flooded plants from the June cohort of *C. rubrum*, the remaining 7 flowering plants produced < 10 seeds, and of the flooded plants fromt the August cohort, the remaining 8 flowering plants did not produce any seeds. **Determined from 5 samples of 100 seeds each of three plants per cohort and treatment; the nested model explained 54% of the seed-weight variation in *C. rubrum* and 93% in *R. maritimus* (see text). Means followed by the same letter are not significantly different (within-species comparisons, Students *t*-test, $p<0.05$).

Discussion

Growth and survival in relation to flooding

Upon submergence, plants of all three species showed the response of reorientation of leaves and branches from prostrate to vertical which is commonly observed in aquatic plants (Ridge 1987) and important in shallow water (Chapter 2). In addition, rosette plants of both *Rumex* spp. exhibited petiole extension of younger leaves (Fig. 3). Survival of longer periods of submergence in the growing season depends on the restoration of shoot-air contact by means of rapid under-water shoot extension in several flooding-tolerant *Rumex* spp. (Voesenek & Blom 1989b; Laan *et al.* 1990; Chapter 7). In *C. rubrum*, young as well as old leaves died during submergence, whereas in both *Rumex* spp., old leaves died first. The latter is of advantage with respect to growth of younger leaves and regeneration of shoots afterwards (Throught & Drew 1980; Jackson & Drew 1984). The higher overall flooding tolerance in *R. palustris* may result from a more efficient use of stored assimilates and/or a relatively high rate of alcoholic fermentation compared with *R. maritimus* (Laan & Blom 1990; Voesenek 1990). The flooding tolerance of all three species was highest in the September cohort (Table 1). The mean temperature of the flood-water was near the base temperature for growth of these species during the last flooding period (see Chapter 3) and this illustrates the important role of metabolic rates in the survival of submergence.

Reproduction in relation to flooding

Plants of *C. rubrum* started to flower at an earlier stage and completed the life cycle in a shorter time in later cohorts (Fig. 2, see also Chapter 3). The inability of plants of *C. rubrum* in the September cohort to produce seeds was probably due to low irradiance and/or insufficient photosynthesis (Kinet, Sachs & Bernier 1985). Flooding hardly reduced the probability of flowering in *C. rubrum* (Table 2). The onset of flowering, however, was not completely independent of size since large plants of *C. rubrum* flowered earlier than small ones (Fig. 2, see also Cumming 1969; Cumming & Seabrook 1985). A large size-requirement in long days, gradually changing to a small one in short days probably results in a fine-tuned trade-off between growth and reproduction in *C. rubrum* (see also Chapter 3).

In both *Rumex* spp., the minimum leaf number which must be achieved for flowering to occur remained constant in later cohorts (Fig. 2, see also Chapter 3). Flooding delayed reaching this stage in both long-day species (Fig. 2) and as a result all flooded plants of *R. palustris* remained vegetative. Flooded plants of *R. maritimus* started to flower after they had initiated approximately the same number of primary leaves as drained plants although their actual size (number and size of living leaves) was much smaller. The age- versus size-dependency of the onset of flowering is the subject of many studies (*e.g.* Werner 1975; Lacey 1986; Klinkhamer, De Jong & Meelis 1987; Blom 1988). In most cases some measure of actual plant size is considered to represent developmental stage. In *R. maritimus*, stage and size are not equivalent in this respect. Flowering in this species is therefore probably not determined by the amount of stored reserves in the tap root, as was suggested for (short-lived) perennials with a size requirement for flowering (Werner 1975; Lacey 1986; Silvertown 1987).

Seed number was maximized at the expense of seed size in flooded plants of *R. maritimus* (Table 4). In addition, the seeds from flooded plants had a lighter colour and were less dormant than seeds from drained plants. This suggests that they lack a hard seed coat, which may be the result of both immaturity and development under wet conditions (Silvertown 1984). This appears to be disadvantageous as it may reduce the longivity of these seeds and/or result in the generation of small seedlings under adverse conditions for germination and establishment. Flooded plants of *C. rubrum* hardly managed to produce seeds, but in contrast to *R. maritimus*, those which did possessed relatively large seeds compared with drained plants (Table 4). Potential seed number is determined early in *C. rubrum* (Cook 1976) and depends, together with seed size, on photoperiod during flower induction (Cook 1975). If the number of flowers is reduced afterwards, overfilling of the seeds is likely to occur (Harper, Lovell & Moore 1970). In *C. rubrum*, the larger seeds from flooded plants as well as from later cohorts of drained plants were less dormant than small seeds (see also Chapter 3; Cumming 1969). Decreased dormancy of the larger seeds which are produced on plants grown in short days is also found in *C. album* and in this species is caused by thinner seed coats (Karssen 1970). A large part of the seed-size variation in *C. rubrum* was not explained by treatment effects (Table 4) and must be ascribed to within-plant seed size variation or seed-polymorphism (Harper, Lovell & Moore 1970).

Flooding tolerance in relation to life history

In unpredictable environments r-selection should be favoured (Stearns 1976; Grime 1979). Advantages of the annual strategy are a high intrinsic rate of increase through fast growth and early reproduction, and survival of adverse conditions by means of persistent seeds in the soil.

Disadvantages are the loss of competitive ability which is probably not very important in the open habitats in river forelands, and the yearly need of establishment microsites (Crawley 1986). In low areas of river forelands, the frequent occurrence of floods allows frequent re-establisment during the growing season but also acts as a stress or disturbance factor for established plants. All three species profited from the advantages of the annual strategy under conditions that allowed early germination and fast growth. In case of later germination and/or disturbance by flooding in the established phase they showed partitioning of resources to different fitness components (Harper 1977; Venable 1984). The strictly annual *C. rubrum* allocated even more resources into seeds than into other structures in later cohorts (Chapter 3). The remarkable plasticity with respect to reproductive parameters in this species was typically expressed between plants (Bradshaw 1965). Seed-polymorphism in *C. rubrum*, however, was only partly regulated by environmental control (Table 4). Within-plant seed size variation may act as a kind of bet-hedging against unpredictable conditions in the habitat (Harper, Lovell & Moore 1970). For example, in *Atriplex triangularis*, small seeds are incorporated into a persistent seed bank and thus ensure long-term population survival, whereas large seeds germinate early and have a better chance of surviving until the reproductive stage (Khan & Ungar 1986). Both *Rumex* spp. allocated more resources into vegetative growth in later cohorts and instead of plasticity in reproductive parameters they showed within-plant plasticity with respect to traits promoting survival, such as the ability to produce different leaf types under flooded and drained conditions. *R. maritimus* flowered more rapidly and was more susceptible to flooding than *R. palustris*. The three species appear to have different features of the opposing strategies of stress tolerators and ruderals which may be found in the most frequently flooded parts of river floodplains (Menges & Waller 1983). *R. palustris* is best capable of maintaining occupation of sites which probably explains its occurrence in associations of marsh species. This species may be described as a stress-tolerant ruderal (*sensu* Grime 1979), while *R. maritimus* and *C. rubrum* possess increasingly ruderal characteristics.

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FLOODING RESISTANCE AND SHOOT ELONGATION IN RELATION TO ENVIRONMENTAL CONDITIONS AND DEVELOPMENTAL STAGE IN RUMEX MARITIMUS AND RUMEX PALUSTRIS

with C.W.P.M. Blom and G.W.M. Barendse

The flooding resistance of *Rumex maritimus* and *Rumex palustris*, two species from frequently flooded areas of river forelands, was studied in a large-scale outdoor experiment. Plants were raised in cohorts and in several stages of development subjected to four weeks of submergence in early summer or in midsummer.
With the exception of seedlings, which were relatively flooding tolerant, plants of both species appeared to depend to a different degree on under-water shoot elongation for the survival of flooding as well as reproductive development afterwards. In *R maritimus*, survival under both conditions of early and midsummer flooding was largely restricted to plants which emerged from the water surface with the bolting shoot. As a consequence, survivors of this species all reproduced in the first growing season and seed output was correlated with stem length immediately after flooding. This course of events was equally important in *R palustris* under conditions of midsummer flooding. In early summer, however, plants of this species were able to survive four weeks of total submergence in the pre-reproductive stage. In these plants, flowering was delayed or postponed to the following season.
Seed-size variation was much larger in *R. maritimus* than in *R palustris* and more clearly related to cohort and treatment effects. Differences between both species in flooding resistance did not seem to be primarily caused by differences in shoot elongation, but by differences in life-history characteristics and tolerance of oxygen deficiency.

Introduction

In river forelands in The Netherlands, *Rumex maritimus* L. and *Rumex palustris* Sm. are typical inhabitants of the lowest areas, such as the shores of former river beds and clay pits (Blom 1990; Blom *et al.* 1990; Van der Sman, Blom & Van de Steeg 1992, Chapter 3 in this thesis). These sites are prone to unpredictable floods during the growing season of which the majority lasts shorter than 2 weeks. Such floods are easily survived by both species of *Rumex* without the restoration of leaf-air contact (Van der Sman, Joosten & Blom 1993, Chapter 4 in this thesis). However, approximately once every 2 years, floods with a duration of more than 3 weeks occur, mostly in early summer and less often in midsummer. Several studies support the view that in order to survive such calamities, plants of both *Rumex* spp. need to restore aerobic metabolism and as a consequence leaf-air contact (Voesenek & Blom 1989b; Laan & Blom 1990; Van der Sman *et al.* 1991, Chapter 7 in this thesis). Rapid shoot elongation in response to flooding is observed in many aquatic and semi-aquatic species (reviewed in: Osborne 1984; Jackson 1985; Ridge 1987). The physiology of the response and especially the role of ethylene is extensively studied in several species, *e.g.* rice (reviewed in Jackson & Pearce 1991) and dock (reviewed in Voesenek *et al.* 1992a,b). Relatively few studies have been published on the functional significance of rapid under-water elongation in relation to both environmental and plant developmental factors. In *Rumex*, differences in elongation response between several species corresponded with their habitat characteristics in river forelands (Voesenek & Blom 1989b; Laan & Blom 1990). In *R. maritimus*, internal aeration was positively correlated with the shoot

part emerging from the water surface (Laan *et al.* 1990). Ridge (1987) used a cost-benefit approach in trying to understand the nature of opposing selective forces in the elongation responses of semi-aquatic species, which commonly grow in air. The ability of rapid elongation is often restricted to certain stages in leaf and plant development in these species. In *R. maritimus*, responses upon submergence varied with the developmental stage of the plant: rosette plants exhibited petiole extension, early bolting plants mostly internode extension, while flowering plants were incapable of further elongation (Chapter 7). A similar pattern was described for *Ranunculus sceleratus* (Samarakoon & Horton 1980). In both *Rumex maritimus* and *R. palustris*, the rate and extent of petiole elongation during submergence appeared to depend on rosette size as well as on mean water temperatures (Chapter 4).

The aim of the present study was to investigate the role of rapid under-water shoot elongation in the flooding resistance of *R. maritimus* and *R. palustris*, as determined by important fitness parameters. Given the unpredictability of floods in river forelands, this study was carried out in a large outdoor experimental set-up. To include both plant developmental as well as environmental factors, plants in different stages of development were subjected to flooding at different times of the year reflecting early-summer and midsummer conditions.

Materials and methods

Plant material and experimental set-up

Cohorts of seedlings of *Rumex maritimus* and *R. palustris* were prepared as described in Chapter 3. Four-day old seedlings were transplanted to separate pots (diameter 16 cm, height 50 cm), filled with a 1:1 mixture (v:v) of river sand and peat. The pots were placed in outdoor basins of water (length x width x depth: 8 x 2.4 x 1 m). Each pot contained seven seedlings, which were thinned to three plants after 2 weeks, and to one after another week. Per cohort and species, thirty pots were prepared, of which the twenty-four most homogeneous ones were selected at the onset of flooding. Drained conditions were provided by a water level of 5-10 cm in the basins. To simulate flooding, they were filled with tapwater to a maximum water height of 90 cm (approximately 40 cm above the soil level in the pots).

The study was performed in 2 consecutive years. In 1988, six cohorts of both *Rumex* spp. were raised (transplanted on 2, 16 and 30 May, 13 and 27 June, and 11 July respectively). The midsummer flooding period of 4 weeks started on 18 July, 11 weeks after the start of the experiment. In the following year (1989), seven cohorts of both *Rumex* spp. were raised (transplanted on 10, 17 and 24 April, 1, 8, 15 and 22 May respectively). In this year, the early-summer flooding period of 4 weeks duration started on 29 May, 8 weeks after the start of the experiment. During the flooding periods, twelve pots per cohort and species were flooded. The other twelve were kept drained throughout the experiment. In 1988, no special measures were taken to adjust water temperatures during the midsummer flooding period. In the last 2 weeks of flooding in 1989, the basins were flushed with cold water during daytimes to keep temperatures at a more realistic level for early-summer conditions in the river forelands. Radiation and air temperature were continuously monitored throughout the study and presented as sums over certain growth periods (see Chapter 3). During flooding periods, water temperatures were measured twice daily (09.00 h and 17.00 h).

Plant measurements

Immediately before and after flooding, the following parameters were measured of all plants: the cumulative number of main leaves > 1 cm and the number of living main leaves, the number of axillary shoots and living axillary leaves, total and petiole lengths of the largest leaf, and the lengths of main and axillary stems. During flooding, plants were observed daily to record leaf and shoot emergence from the water surface as well as the initiation of adventitious roots.

The reproductive development of all surviving plants was recorded throughout the study period. The following stages were distinguished: vegetative (rosette), bolting, inflorescence present, early flowering, out of flower, fruits ripened (*R. maritimus*: golden brown; *R. palustris*: dark red brown). In the results, 50% values per cohort and treatment of these stages are presented. At the end of the experiment, final stem lengths of all plants were measured, as well as the internode lengths of main and axillary stems of five plants per species, cohort and treatment.

Seeds were collected per plant in November and total seed output was weighed after several times of cleaning and sieving. Of five drained plants and all flooded plants per cohort and species, seeds were collected from main and axillary stems separately. The mean weight of fifty seeds (without perianths) was determined of a seed mixture from five drained plants per species, cohort and treatment. In the case of flooded plants, a distinction was made upon flooding response (see results section) and seed mixtures of at least three plants per flooding response type were taken. From each of these mixtures, sixteen samples of fifty seeds were weighed (without perianths). Because of the small sample sizes, this procedure was repeated, which resulted in an interval of several months between the first and last weighings. The two weighing periods were treated as blocks in the statistical analysis of these results. From these data, total seed number was estimated of all reproductive plants. Seed germination was tested with the same samples under constant and fluctuating tempratures in the light and in darkness, as described in Chapter 4.

Statistical analysis

The General Linear Models procedure from the SAS statistical package was used for unbalanced analysis of variance (SAS Institute Inc. 1985). Multiple comparisons of means were made with Bonferroni *t*-tests. Pearson's rank correlation coefficients were determined to indicate correlations.

Results

Growth and survival in relation to shoot emergence

Four responses with respect to shoot growth during flooding were distinguished: (i) emergence with the upper part of the bolting shoot before the end of the flooding period; (ii) emergence with one or more leaf tips only, within 1 (*R. maritimus*) or 2 weeks (*R. palustris*), and maintenance of shoot-air contact afterwards; (iii) emergence of leaf tips as under (ii), but leaf collapse in the third week resulting in resubmergence; (iv) submergence throughout the flooding period. These responses appeared to be important with respect to survival as well as reproductive development afterwards. Table 1 summarizes several plant parameters per cohort of both species immediately before the start of the flooding treatments in relation to temperature

and radiation sums. Between 17 and 24 April 1989, temperatures were very low which delayed the development of plants in the two earliest cohorts, especially of *R. palustris*. Low temperatures also caused within-cohort differences, *e.g.* with respect to the number of axillary leaves (Table 1), which had consequences for reproductive output (next paragraph). At the onset of flooding, all plants were submerged with the exception of a number of plants of both species from the 2-and 16-May cohorts of 1988, which emerged with the top part of the bolting shoot.

Seedlings represented a special case with respect to survival of total submergence (Table 2). In both species and in both years, plants of 1 week old possessing only cotyledonary leaves and a first main leaf of 1 mm at the onset of flooding, performed better than plants beyond the cotyledonary stage, *e.g.* 2 weeks and older. Growth of the first and second primary leaves (up to several mm) was observed under water until the third week of flooding, and plants either died in the fourth week of flooding or shortly afterwards.

Table 1. Plant parameters of *Rumex maritimus* and *Rumex palustris* at the onset of flooding (18 July 1988 and 29 May 1989) in relation to temperature and radiation sums (mean±1SE, n=12).

Cohort	Age (weeks)	Temperature sum (degree-days)*a*	Radiation sum (Jcm^{-2})	Cumulative (and living) no. of main leaves (cm)	
				R. maritimus	*R. palustris*
2 May 1988	11	481	116 000	27.1(17.8)±0.3	26.4(17.4)±0.2
16 May 1988	9	389	90 000	22.7(14.7)±0.6	20.1(12.0)±0.1
30 May 1988	7	309	67 000	14.3(11.3)±0.3	13.6(10.7)±0.4
13 June 1988	5	213	44 000	8.7(8.7)±0.2	8.3(8.2)±0.1
27 June 1988	3 *b*	148	27 000	3.3(3.3)±0.1	3.0(3.0)±0.1
11 July 1988	1 *c*	41	6000	0 *d*	0 *d*
10 April 1989	7	212	85 000	10.4(10.3)±0.1	8.8(8.3)±0.1
17 April 1989	6	200	78 000	9.6(9.3)±0.1	8.8(8.3)±0.1
24 April 1989	5	199	71 000	8.9(8.6)±0.1	8.3(8.1)±0.1
1 May 1989	4	193	61 000	7.3(7.3)±0.1	6.9(6.9)±0.1
8 May 1989	3	159	47 000	5.2(5.2)±0.1	5.0(5.0)±0.0
15 May 1989	2 *b*	136	34 000	2.2(2.2)±0.1	2.3(2.3)±0.0
22 May 1989	1 *c*	68	18 000	0 *d*	0 *d*

Note: nd=not determined; *a* Base temperature 10 °C; *b* n=36 (three plants per pot); *c* n=84 (seven seedlings per pot); *d* First real leaf *c.* 1 mm; *e* Bolting axillary shoots only. In the first three cohorts of 1988, plants were in the bolting stage with mean main stem length: 45.6 cm, 39.8 cm, and 9.3 cm respectively in *R. maritimus*, and 57.3 cm,31.7 cm, and 4.7 cm respectively in *R. palustris*.

In 1988, water temperatures ranged from 15/20 °C (09.00 h/17.00 h) in the first week of flooding up to 20/25 °C in the last 2 weeks. Both *Rumex* spp. responded in a rather similar way to this midsummer flooding treatment. Survival was, with the exception of seedlings, restricted to plants which had emerged with the bolting shoot before the end of the flooding period (Table 2). All these plants were beyond the rosette stage at the onset of flooding (Table 1). Plants from the 30-May cohort of 1988 emerged with one or more leaf tips from large rosette leaves before shoot tips emerged. In the furthest developed plants of *R. maritimus* from the 2-May cohort, neither main nor axillary stems were significantly increased in length after flooding (Fig. 1, Table 3; see also Chapter 7). In several flowering plants of this species which remained almost completely submerged, however, small axillary shoots from higher main and axillary stem nodes emerged from the water surface. Rosette plants of both species in the June cohorts of 1988 were unable to reach the water surface in spite of a threefold increase in leaf length in the first week of flooding. Severe chlorosis was observed from approximately 10 days onwards and plants of *R. maritimus* from these cohorts died in the third week of flooding and those of *R. palustris* in the fourth week.

Table 1 continued:

Axillary shoot no.		Axillary leaf no.		Maximum leaf length (cm)	
R. maritimus	*R. palustris*	*R. maritimus*	*R. palustris*	*R. maritimus*	*R. palustris*
3.7±0.3 *e*	3.7±0.2 *e*	nd	nd	22.3±0.7	23.8±0.2
4.8±0.3 *e*	3.8±0.3 *e*	nd	nd	26.1±0.7	24.6±0.4
6.2±0.1	9.4±0.2	33.2±0.1	46.3±1.2	25.6±0.7	24.8±0.3
5.6±0.1	5.2±0.1	23.7±0.3	22.5±0.3	10.4±0.8	12.6±0.2
-	-	-	-	3.6±0.1	1.9±0.1
-	-	-	-	-	-
6.4±0.1	6.4±0.1	31.3±1.0	25.1±0.8	16.6±0.6	12.4±0.9
6.1±0.2	5.1±0.2	25.0±0.9	17.3±1.3	15.4±0.7	13.0±0.3
5.8±0.1	5.4±0.1	24.4±0.8	20.6±0.5	13.4±0.5	11.7±0.3
4.2±0.1	3.9±0.1	14.1±0.3	11.4±0.4	10.0±0.3	8.9±0.2
2.2±0.1	2.0±0.0	4.5±0.3	4.2±0.2	4.5±0.2	4.8±0.2
-	-	-	-	1.2±0.1	1.5±0.1
-	-	-	-	-	-

Table 2. Percentage survival in *Rumex maritimus* and *Rumex palustris* after 4 weeks of flooding (40 cm above the soil) in relation to type of response: (i/ii) emergence with part of the reproductive shoot / or with only 1-3 leaf blades; (iii) emergence but leaf collapse before the end of the flooding period; (iv) submergence throughout the flooding period; Number of plants out of 12 which exhibited this response between brackets.

Cohort	Response type					
	(i/ii)		(iii)		(iv)	
	R. maritimus	*R. palustris*	*R. maritimus*	*R. palustris*	*R. maritimus*	*R. palustris*
2 May 1988	100(10)	100(12)	-	-	0(2)	-
16 May 1988	100(12)	100(12)	-	-	-	-
30 May 1988	100(12)	100(12)	-	-	-	-
13 June 1988	-	-	-	-	0(12)	0(12)
27 June 1988	-	-	-	-	0(36*)	0(36*)
11 July 1988	-	-	-	-	2(84**)	0(84**)
10 April 1989	100(11)	100(5)	0(1)	100(6)	-	100(1)
17 April 1989	100(7)	100(2)	80(5)	100(8)	-	50(2)
24 April 1989	-	-	100(1)	100(1)	0(11)	100(11)
1 May 1989	-	-	-	-	0(12)	75(12)
8 May 1989	-	-	-	-	0(12)	0(12)
15 May 1989	-	-	-	-	0(36*)	0(36*)
22 May 1989	-	-	-	-	1(84**)	1(84**)

*Three plants per pot; **Seven seedlings per pot; more seedlings survived but died several weeks afterwards.

During the early-summer flooding treatment of 1989, water temperatures gradually rose from 12/15 °C up to 17/22 °C (09.00 h/17.00 h). Although none of the plants had started bolting at the onset of flooding, they survived the treatment in several cohorts (Table 2). However, most surviving plants of *R. maritimus* had showed the same response of shoot emergence during flooding as in 1988. The remaining survivors had at least temporarily reached the water surface and were bolting by the end of the flooding period (Fig. 1). The first two internodes of the main stem of all plants of *R. maritimus* which emerged with the shoot were twice as long as those of drained plants (data not shown); following internodes were of the same length. Differences between the responses to the midsummer (1988) and early-summer (1989) flooding treatments were much larger in *R. palustris*. More and smaller plants of this species survived the early-summer treatment without emergence of the bolting shoot. As much as 50% of all surviving plants of *R. palustris* in 1989 had even remained completely submerged (Table 2). Only a small number of plants had already initiated a stem at the end of flooding (Fig. 1). At the end of the growing season all internodes of flooded plants were smaller than parallel internodes of drained plants. Petiole extension was apparent in plants of both species which had emerged from the water surface but most strongest in those which had not already emerged with the bolting shoot (Fig. 1).

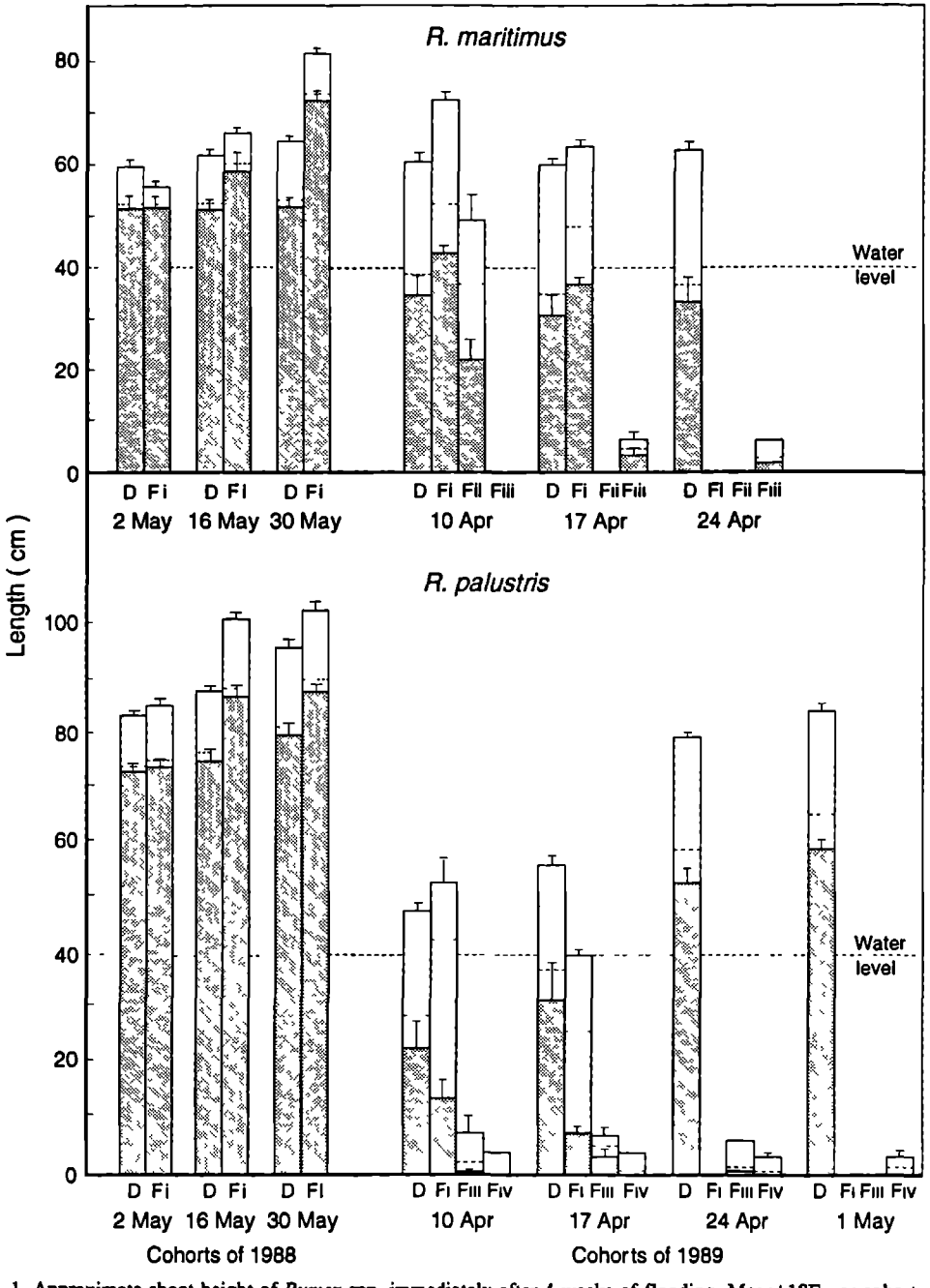


Fig. 1. Approximate shoot height of *Rumex* spp. immediately after 4 weeks of flooding. Mean±1SE per cohort, treatment (D: Drained; F: Flooded) and flooding response (i-iv: see Table 2, also for *n*). Shaded area of bars: main stem length; open area: length of longest leaf (leaf-petiole junction indicated).

Table 3. Plant parameters of *Rumex maritimus* and *Rumex palustris* immediately after flooding (15 August 1988 and 26 June 1989). Only those cohorts are presented in which plants survived the flooding treatment (with the exception of seedlings, see Table 2). Mean±1SE, *n* as shown per cohort and treatment.

Cohort	Age (weeks)	Treatment	*n*		Cumulative (and living) no. of main leaves (>1 cm)	
			R. m.	*R. p.*	*R. maritimus*	*R. palustris*
2 May 1988	15	Drained	12	12	32.5(10.3)±0.8	37.8(20.0)±0.3
		Flooded	10	12	33.2(8.1)±0.7	38.5(21.4)±1.1
16 May 1988	13	Drained	12	12	30.7(9.7)±0.8	36.1(23.6)±0.6
		Flooded	12	12	30.7(8.5)±0.7	37.7(22.7)±1.0
30 May 1988	11	Drained	12	12	32.0(14.8)±0.6	39.2(18.6)±0.6
		Flooded	12	12	31.2(12.1)±0.8	35.3(22.4)±0.8
10 April 1989	11	Drained	12	12	27.3(19.9)±1.4	18.7(10.8)±0.9
		Flooded	11	12	20.5(10.2)±1.2	13.8(3.1)±0.6
17 April 1989	10	Drained	12	12	23.5(16.1)±1.6	22.0(15.4)±1.6
		Flooded	11	11	17.2(7.2)±1.3	12.6(1.5)±0.3
24 April 1989	9	Drained	12	12	24.5(17.4)±1.4	27.6(19.5)±0.7
		Flooded	1	12	11(1)	11.7(1.6)±0.2
1 May 1989	8	Drained	12	12	27.3(20.6)±1.2	28.3(20.4)±0.3
		Flooded	0	9	-	8.8(1.1)±0.2

Note: nd=not determined; *Number of bolting shoots only; **n=11 (one plant remained vegetative).

Adventitious roots were only initiated in plants which emerged from the water surface, both in 1988 and in 1989. A dense mat of roots with tiny laterals was formed at the stem base of all these plants (*cf.* during waterlogging in Van der Sman, Van Tongeren & Blom 1988, Chapter 2 in this thesis). Bolting plants also initiated a much smaller number of thick roots from higher submerged stem nodes. Adventitious roots dried out immediately after removal of the flood-water. Also after flooding, extended leaves and stems were brittle and more easily damaged than those of drained plants.

Reproduction in relation to shoot emergence

In 1988, with the exception of seedlings which are not further discussed, all plants which survived summer flooding were already bolting before the onset of the treatment, and further reproductive development was hardly delayed (Fig. 2).

In the first two cohorts of 1989, plants of *R. maritimus* bolted during the flooding treatment without delay compared with drained plants (Fig. 2). The development of the inflorescence and fruit ripening, however, were considerably delayed due to flooding.

Table 3 continued:

Axillary shoot no.		Axillary leaf no.		Axillary stem length (cm)	
R. maritimus	*R. palustris*	*R. maritimus*	*R. palustris*	*R. maritimus*	*R. palustris*
4.0±0.3*	3.8±0.1*	nd	nd	162±11	226±8
3.8±0.3*	3.9±0.1*	nd	nd	173±16	271±9
5.6±0.1*	4.2±0.2*	nd	nd	200±8	249±12
5.3±0.2*	4.0±0.2*	nd	nd	285±11	351±16
4.4±0.3*	3.7±0.3*	nd	nd	182±10	231±25
3.8±0.2*	2.3±0.3*	nd	nd	237±14	162±25
7.9±0.4	9.3±0.6	96.6±8.3	52.2±4.7	125±23	56±17**
4.5±0.8	0.2±0.1	7.0±1.7	0.2±0.1	-	-
8.4±0.5	7.6±0.8	90.8±9.9	54.3±9.5	118±28	98±26
2.9±0.8	0.2±0.1	4.0±1.1	0.2±0.1	-	-
8.3±0.5	8.8±0.2	81.9±8.7	85.0±4.1	117±26	182±11
-	0.3±0.2	-	0.3±0.2	-	-
7.2±0.3	8.0±0.0	87.4±6.3	76.0±2.9	139±16	162±10
-	-	-	-	-	-

In *R. palustris*, bolting was delayed until after the early summer flooding treatment of 1989 (Fig. 2). As much as 25% of all surviving plants of this species even postponed flowering until the following growing season. Flooded plants of *R. palustris* from the 1-May cohort bolted at the end of July after they had initiated twelve to fifteen main leaves. By that time, plants from the April cohorts which remained vegetative had initiated several more main leaves and were considerably larger than those from the 1-May cohort.

In the first two cohorts of 1988, seed output was correlated with stem length immediately after flooding in *R. maritimus* (Table 4). In these cohorts, plants of *R. maritimus* showed clear differences in the shoot part emerging from the water surface. In cohorts of either species in which all plants had emerged with a large part of the shoot, there was no such correlation (Table 4). In both species, however, the seed output of flooded plants from the 30-May cohort of 1988 was relatively low (Fig. 3). In this cohort, the number of axillary stems was significantly reduced in both species, whereas both the lengths of main and axillary stems was much increased by flooding (Fig. 1, Table 3).

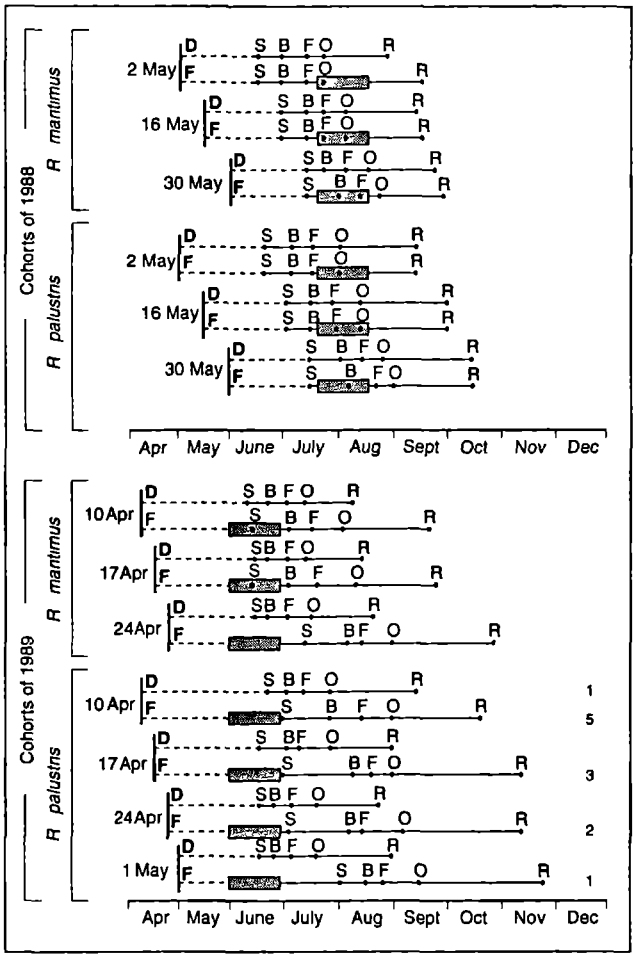


Fig. 2. Reproductive development of *Rumex* spp. in outdoor plots in relation to flooding (shaded blocks). Presented are the 50% values per cohort and treatment (D: drained; F: flooded) of the time of S: stem elongation, B: appearance of flower buds, F: early flowering, O: out of flowers, R: ripening of fruits. Dotted lines: one ore more plants (number indicated) remained vegetative (n=12 or less, see Table 2).

In 1989, differences between both species in seed output, presented per cohort in Fig. 3, were even more clearly related to the earlier flooding response (Table 4). In *R. maritimus*, most seeds were produced by the plants which emerged with the shoot in the 10- and 17-April cohorts. These plants produced the same mean amount of seeds on the main stem as drained plants (data not shown). Only a very small part of the total seed output of the flooded plants originated from small axillary stems (up to 10 cm) which appeared after the flooding treatment. In both species, plants which collapsed during the flooding treatment produced only very small numbers of seeds. In the April cohorts of 1989, there was a larger within-cohort variation in plant size in both species, probably as a result of the low temperatures in April (Table 1). In these cohorts, there was a positive correlation between estimated above-ground biomass at the onset of the flooding treatment and seed output (Table 4). In flooded plants in these and other cohorts,

however, seed output did not correlate well with estimated biomass. Positive correlations of seed output with leaf and/or stem length immediately after flooding were therefore not simply due to between-plant differences in biomass that were already present before flooding.

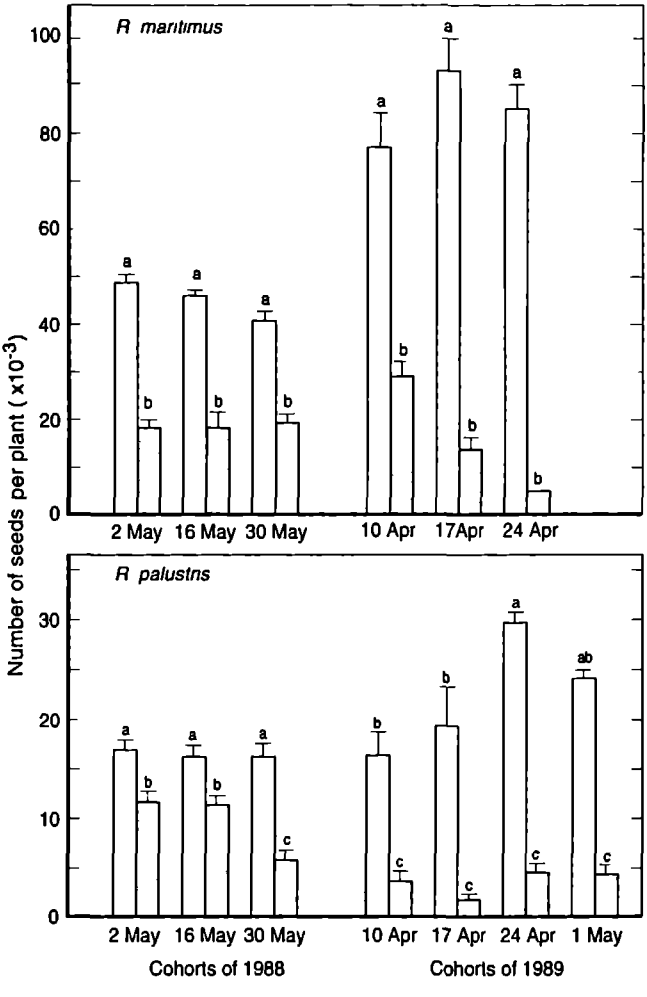
The mean fifty-seed weight varied between 24 mg and 28 mg in *R. palustris* and between 8 mg and 13 mg in *R. maritimus*. In *R. maritimus*, cohort and treatment effects were more important than in *R. palustris* (Table 5). The block effect was probably caused by differences in relative humidity between both weighing periods. There were small but significant effects of flooding response in both species. In *R. maritimus*, plants which had emerged with the shoot had larger seeds than drained plants and all other flooded plants had smaller seeds. There were no clear changes observed in germination characteristics which could be attributed to either treatment or cohort effects (data not shown).

Table 4. Within-cohort correlations (Pearson correlation coefficient) between seed output (number of seeds per plant) and plant parameters before and after flooding in *Rumex* spp. (n=12 or less, see Table 2 and Fig. 2).

Cohort	Estimated biomass before flooding *a*		Maximum leaf length after flooding		Main stem length after flooding	
	Drained	Flooded	Drained	Flooded	Drained	Flooded
R. maritimus						
2 May 1988	0.22 NS	-0.26 NS	nd	nd	0.15 NS	0.85 ***
16 May 1988	-0.43 NS	0.23 NS	nd	nd	0.59 *	0.77**
30 May 1988	0.11 NS	0.02 NS	nd	nd	-0.03 NS	0.04 NS
10 April 1989	0.74 **	0.39 NS	0.85 ***	0.30 NS	-0.14 NS	0.82 **
17 April 1989	0.72 **	0.69 *	0.50 NS	0.85 ***	0.47 NS	0.83 ***
R. palustris						
2 May 1988	-0.36 NS	-0.29 NS	nd	nd	-0.14 NS	0.13 NS
16 May 1988	-0.46 NS	0.56 NS	nd	nd	0.03 NS	-0.16 NS
30 May 1988	-0.04 NS	0.59 *	nd	nd	-0.44 NS	0.02 NS
10 April 1989	0.92 ***	0.18 NS	0.69 *	0.95 **	0.88 ***	0.76 *
17 April 1989	0.78 **	0.30 NS	0.86 ***	0.80 *	0.97 ***	0.86 **
24 April 1989	0.57 *	-0.10 NS	0.87 ***	0.22 NS	0.56 *	-
1 May 1989	0.35 NS	-0.27 NS	0.26 NS	0.47 NS	0.23 NS	-

Note: nd=not determined; *a* Number of living leaves x maximum leaf length (see Chapter 2); Statistical significance: NS=not significant; *p<0.05; **p<0.01; ***p<0.001.

Fig. 3. Mean number of seeds (±1SE, n=12 or less, see Table 2 and Fig. 2) in *Rumex* spp. in outdoor plots. Per cohort and treatment (open: drained; shaded: flooded as indicated in Fig. 2) Means indicated with the same letter are not significantly different (Bonferroni t-test, $p<0.05$). Comparisons within species and years only.



Discussion

Different elongation responses occur upon submergence of plants of *Rumex* in different stages of the life cycle (Chapter 7). In the present study, the impact of these responses on survival and reproduction after 4 weeks of flooding was examined. The role of petiole and internode extension varied with flooding conditions and plant developmental stage in both *R. maritimus* and *R. palustris*. In the seedling stage, elongation processes were unimportant as expected. However, seedlings of both species were better able to survive submergence than older juveniles, in early summer as well as in midsummer (Table 2). The relatively high flooding tolerance of seedlings of *R. palustris* and *R. crispus* was attributed to both morphological and metabolical features (Voesenek *et al.* 1992c).

Table 5. Analysis of variance (GLM-procedure, SAS Institute Inc. 1986) of mean 50-seed weight in *Rumex maritimus* and *Rumex palustris* with block, cohort (nested within block), flooding treatment (nested within block), and flooding response (nested within treatment) as class variables. For all sources of variation, *d.f.*, *F*, *p*, and percentage of total sum of squares (Var.) are presented. Per class variable, the general order of decreasing seed weight is presented.

Source of variation	*d.f.*	*F*	*p*	%Var.	General order *a*
R. maritimus					
Block	1	113	***	4	2>1
Cohort (Block)	10	200	***	65	30M>16M>13J>17A>10A
Treatment (Block)	2	182	***	12	Flooded>Drained
Response (Treatment)	4	16	***	2	(i)'88>(i)'89>(d)>(iii)>(ii)
Cohort x Treatment	3	35	***	3	
Model				86	
R. palustris					
Block	1	69	***	8	2>1
Cohort (Block)	14	18	***	30	13J>10A>24A>1M>17A>30M>16M>2M
Treatment (Block)	2	8	***	2	Flooded>Drained
Response (Treatment)	3	7	***	3	(ii)>(iv)>(iii)>(d)>(i)
Cohort x Treatment	4	8	***	4	
Model				47	

a Weighings in block 2 occurred several months later than in block 1; Cohorts: A=April, M=May, J=June; Responses: (i)'88 refers to response type (i) of 1988 etc. as described in Table 2, d=drained. Statistical significance: ***$p<0.001$.

Survival of rosettes of both species depended on flooding conditions and on the ability to emerge from the water surface. Midsummer floods occur occasionally in the river forelands. Under these conditions, vegetative plants of both species were unable to survive 4 weeks of submergence (Table 2). Under conditions of early-summer flooding, several cohorts of rosettes of *R. palustris* were able to survive 4 weeks of submergence which suggests that this species is less dependent on oxygen availability and as a consequence on elongation processes than *R. maritimus* (*cf. R. crispus* in Laan & Blom 1990). Mean water temperatures during flooding are probably of crucial importance in this respect. The temperatures of the early-summer flooding treatment are more typical for the prevailing conditions in river forelands. Relatively high temperatures promoted shoot extension in both species, while low temperatures resulted in less reduction of biomass, probably due to reduced metabolic rates (see Chapter 4). In the present study, collapse of extended leaves occurred earlier during the midsummer than during the early-summer treatment. Jackson *et al.* (1987) argued that when the water is too deep to get and maintain foliage above the water, greater elongation and the associated chlorosis of older leaves may result in collapse and wilting of the shoot when water levels recede. Conform this argument, the role of leaf elongation in the rosette stage appeared to be limited in both *Rumex* spp. and leaf collapse a severe threat, especially under midsummer conditions. In *R. maritimus*,

survival beyond the seedling stage was highly dependent on regaining and maintaining shoot-air contact, and in order to do this stem extension had to proceed soon after leaf emergence. In rice, under-water growth was compared between different cultivars (Keith, Raskin & Kende 1986). The duration of the bolting stage, during which internodal growth was promoted by submergence, was much longer in deepwater rice than in other cultivars, and this developmental feature provided the deepwater character rather than a larger short-term elongation capacity of the internodes. Differences between both *Rumex* spp. in elongation response also appeared to be largely developmental. As a consequence of survival being restricted to plants which emerged with the bolting shoot, surviving plants of *R. maritimus* were exclusively annual. In *R. palustris*, on the other hand, early-summer flooding delayed bolting as well as further reproductive development. This was only partly caused by a delay in reaching a certain plant stage or size which is a common prerequisite for flowering in biennial plants (*e.g.* Lacey 1986; Klinkhamer, de Jong & Meelis 1987).

In bolting plants of both species, there was a tendency to reduce the number of stems and to increase stem length through under-water internode extension during flooding (Table 3). In general, reduction in seed output compared with drained plants was less after flooding in those plants of either species which emerged from the water surface with a large part of the reproductive shoot (see also Chapter 7). The relation between plant height and seed output was especially clear in *R. maritimus* (Table 4), probably because the water height of 40 cm was relatively high for this species. Above a certain plant height seed output was reduced again, *e.g.* in the 30 May cohort of 1988. This may have been partly due to a significant reduction in axillary stem number after flooding in this cohort, since seed output was correlated with number and size of stems in these species under drained conditions (Chapters 3 and 4). However, these results probably also indicate the metabolic cost of stem elongation and a trade-off between the productions of stems and seeds. There appeared to be a certain optimum stem size in both species above which further elongation was disadvantageous. This size is probably dependent on water level, and in river forelands, where water levels may exceed 40 cm during at least part of longer flooding periods, *R. palustris* has the dual advantage over *R. maritimus* of longer stems and of the ability to survive submergence for longer periods. Within cohorts, the seed output of flooded plants was correlated with shoot length after flooding rather than with estimated biomass before flooding (Table 4). This suggests that under-water elongation is not completely biomass dependent. The developmental stage of leaves and internodes at the onset of submergence may be crucial for their final lengths (L.A.C.J. Voesenek and A.J.M. van der Sman, unpublished).

Plants of *R. palustris* from the 24-April and 1-May cohorts of 1989 seemed to profit from remaining submerged in terms of seed production. In these plants, reproduction was delayed until after several weeks of recovery (Fig. 2). This resulted in a relatively large seed output compared with plants from the 17–April cohort, most of which emerged and collapsed afterwards.

Mean seed size was smaller and seed-size variation larger and more clearly related to environmental conditions in *R. maritimus* than in *R. palustris*. In *R. maritimus*, seeds tended to be larger in later-raised cohorts, in flooded plants in general, and more specifically in those plants which had emerged with the reproductive shoot from the water surface. These were probably all examples of overfilling of seeds where seed number was already determined (Harper, Lovell & Moore 1970). In the strictly annual species *Chenopodium rubrum*, which occurs in the same habitat types in river forelands as both *Rumex* species, seed size was related

to cohort and flooding effects in the same manner as in *R. maritimus* (Chapter 4). *C. rubrum* was unable to survive 4 weeks of submergence in experimental plots (A.J.M. van der Sman, unpublished). *R. maritimus* takes an intermediary position between *C. rubrum* and *R. palustris* with respect to earliness of flowering and seed-size variability on the one hand and developmental and metabolical features conveying flooding tolerance on the other (see also Chapter 4). These features appear to be components of the opposing strategies of ruderals and stress tolerators (*sensu* Grime 1979) which both may be found in the most frequently flooded areas of river floodplains (Menges & Waller 1983). Elongation responses in *Rumex* seem to provide a rather opportunistic strategy as was also suggested for *Ranunculus sceleratus* (Ridge 1987). The higher flooding resistance of *R. palustris* compared with *R. maritimus* is provided by the ability to tolerate longer periods of oxygen deficiency and to delay flowering rather than the ability to bolt and exhibit internode extension.

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LIFE HISTORIES OF RUMEX MARITIMUS AND RUMEX PALUSTRIS IN RIVER FORELANDS; THE RELATIONSHIP BETWEEN UNDER-WATER SHOOT ELONGATION IN EARLY SPRING AND REPRODUCTIVE DEVELOPMENT IN THE FOLLOWING SEASON

with H.M. van de Steeg and C.W.P.M. Blom

The occurrence of different life histories in *Rumex maritimus* and *Rumex palustris* was studied in relation to flooding regimes, both in their natural habitats in river forelands and in experimental plots. The annual strategy occurred in *R. maritimus* under rather exceptional conditions of early retreat of winter high-waters and only shallow flooding in the growing season. The biennial strategy was most common in both species. In *R. palustris*, short-lived perennality was also observed, not through delay of flowering but by means of vegetative proliferation after flowering.
Survival of several months of winter submergence was highest in large plants of both species, which had not flowered in the previous year. Under experimental conditions, under-water shoot elongation resulted in emergence of these plants in early spring. In their natural habitats, this course of events which relieves oxygen stress may enhance survival of winter and early summer floods and accelerate reproductive development in the following season.
R. palustris was more tolerant of total submergence than *R. maritimus*. The role of different life histories in both species is discussed in relation to their different tactics of flooding resistance.

Introduction

Outdyke areas along major rivers in The Netherlands, so-called river forelands, are characterized by extremely fluctuating water levels (Blom 1990; Blom *et al.* 1990). Unpredictable peaks in water discharge by the river are caused by precipitation peaks in the Rhine catchment area. These are superimposed on the more predictable yearly pattern of relatively high water levels in winter and spring and low levels in summer and autumn. *Rumex palustris* Sm. and *Rumex maritimus* L. inhabit some of the most frequently flooded areas of river forelands such as mud flats and sand and clay pits (*e.g.* Van der Sman, Blom & Van de Steeg 1992, Chapter 3 in this thesis). In frequently flooded areas of river floodplains, stress tolerators as well as ruderals (*sensu* Grime 1979) are found (Menges & Waller 1983). Fitter & Hay (1981) distinguished three sets of tactics to cope with stresses of different kinds: (i) avoidance, (ii) amelioration, and (iii) tolerance. The two *Rumex* spp. under study show elements of each of these approaches. Avoidance of the adverse conditions of winter and spring flooding may occur by means of a supply of persistent seeds in the soil and specialized germination characteristics (Roberts & Boddrell 1985; Voesenek & Blom 1992a,b). Both species may indeed adopt the annual life history. If early retreat of winter and spring high-waters allows spring germination and there is no disturbance by flooding during the vegetative stage, plants of *R. palustris* and *R. maritimus* complete their life-cycle in one growing season (Chapter 3). In order to flower in the year of germination, they must reach a mature developmental stage before a certain photoperiod has passed. This "critical" photoperiod is longer in *R. palustris* than in *R. maritimus*. The frequently occurring spring and summer floods in their habitats in river

forelands, however, may cause both a delay in germination as well as a delay in reaching the mature stage (Van der Sman, Joosten & Blom 1993, Chapter 4 in this thesis).

Upon submergence in the growing season, both species respond with under-water shoot elongation in order to regain shoot-air contact (Laan & Blom 1990; Voesenek 1990; Van der Sman *et al.* 1991, Chapter 7 in this thesis). This is an example of amelioration: oxygen is transported from the emerging shoot to the root system and the adverse condition of anaerobiosis is avoided (Laan *et al.* 1990). Internal aeration is further enhanced through the initiation of adventitious roots after shoot emergence. *R. maritimus* appears to depend more upon this strategy than *R. palustris*. The latter species is best capable to survive under different regimes of total submergence, suggesting real tolerance of anaerobiosis (Chapters 4 and 5). Ethanolic fermentation may play an important role in this tactic of anoxia tolerance in *R. palustris* (Voesenek *et al.* 1992c).

In plants of both species which started to flower nearer the critical photoperiods for flowering, less axillary shoots bolted and flowered than in earlier flowering plants (Chapter 3). After flowering of the main shoot, vegetative axillary shoots were able to survive in experimental plots until the following growing season. At the end of the season, several cohorts of vegetative plants of both species are found in river forelands which must survive winter and spring flooding in order to reproduce in the following growing season. During the winter months, peak water levels up to several metres above the soil commonly occur. However, in early spring, water levels often recede to less than 1 m, which may be bridged by elongating shoots of both *Rumex* species. This strategy of survival in both species was described for other, less dynamic wetland systems (Hejny 1960).

This study presents an inventory of field observations on the occurrence of different life histories in *R. maritimus* and *R. palustris* in relation to flooding regimes in river forelands. The biennial and triennial life histories of the species were studied in outdoor experimental plots. The main experiment was designed to study the significance of under-water shoot elongation in early spring for the survival of prolonged winter flooding and reproductive development in the following season. The relative importance of different life histories in both species is discussed in relation to the three mentioned tactics of avoidance, amelioration and tolerance (*sensu* Fitter & Hay 1981), to cope with extremely fluctuating water levels.

Materials and methods

Field observations

All field observations on *R. maritimus* and *R. palustris* were made in a clay pit and on the shore of an old river bed in the Kekerdomse Waard near Nijmegen, The Netherlands. The main results were obtained from two series of observations made over the periods 1976-80 and 1987-89, inclusive, on the river-bed shore, the most important habitat type of both species in river forelands. Data from relevés and field studies are summarized with respect to the life histories of both *Rumex* species in relation to flooding characteristics in these years. In the first period there was a certain delay in the subsidence of floods relative to river water level due to the presence of a natural river levee between the river and the old river-bed shore. This levee was dug away before the second period, and height relative to river water level was probably a more direct measure of flooding regimes in the period 1987-89.

Outdoor flooding experiments

In 1987, four cohorts of plants of *R. maritimus* and *R. palustris* were raised in outdoor plots in accordance with four flooding periods as described in Chapter 4. Per cohort and species, twenty plants were kept drained throughout the first growing season and another twenty were subjected to 10-day flooding periods. The first (May) cohort was flooded four times, the second (June) three times, the third (August) twice and the last (September) once. Nearly all plants survived these flooding treatments (Chapter 4). In *R. palustris*, only the drained plants from the May cohort flowered (95%) in 1987. At the end of the growing season, 50% of these plants still possessed one or two vegetative shoots in rosette leaf axils. In *R. maritimus*, flowering occurred in the May and June cohorts of 1987. All drained plants flowered in these cohorts and part of the flooded plants. After flowering, approximately 50% of the plants (both drained and flooded) from the June cohort of *R. maritimus* still possessed vegetative axillary shoots. Four randomly chosen plants per species, cohort and treatment were harvested in December to provide data on mean plant size at the onset of the winter treatment (Fig. 1). The remaining sixteen plants (or less in flowering cohorts) per species, cohort and treatment were randomly divided over two winter treatments. Eight plants were flooded from 6 January to 2 May 1988, the other eight were kept drained in this period. Water levels during the flooding period were approximately 40 cm above the soil (see Chapters 4 and 5). Water temperatures were measured twice daily (09.00 h and 17.00 h). Plants were studied during the winter treatment and during the following growing season under drained conditions. Air temperatures were monitored throughout the study period.

In 1988, two cohorts of both *Rumex* spp. were raised in the same manner as in the previous year, one in June and one in July with twelve plants per species and cohort (see Chapters 3 and 5). These were kept drained up to January 1989 and flooded from January until April 1989, together with several plants of *R. palustris* from the May, June and August cohorts of 1987 which still possessed vegetative axillary shoots after flowering in 1988. In the early summer of 1989, all these plants were randomly divided over two treatments: 4 weeks of early-summer flooding from 29 May to 26 June, and drained throughout the growing season of 1989. This experiment served to compare the flooding resistance of biennial and triennial plants with that of annual plants, which was studied previously (Chapters 4 and 5).

Plant measurements during and after winter flooding

The numbers of main and axillary leaves, and maximum leaf size (length and width) were measured at monthly intervals (not during submergence). Development of shoot parts emerging from the water surface was recorded of all flooded plants. Immediately after flooding, stem length and leaf and petiole lengths were measured of all surviving plants. The following stages of reproductive development were distinguished: bolting, appearance of the inflorescence, flowering, out of flower, and ripening of fruits (*R. maritimus*: golden brown; *R. palustris*: dark red brown). Ripe seeds including perianths were harvested in August (May to August cohorts of 1987) and September 1988 (September cohort of 1987), and in August 1989 (all cohorts of 1987 and 1988). Total seed output was determined per plant after leaf and soil particles were removed.

Samples were taken from seed mixtures of several plants of *R. palustris* from the August cohort of 1987. In 1988, five plants per treatment were used (two summer and two winter treatments) and in 1989, three plants per treatment (two summer treatments in 1989). Up to the summer of

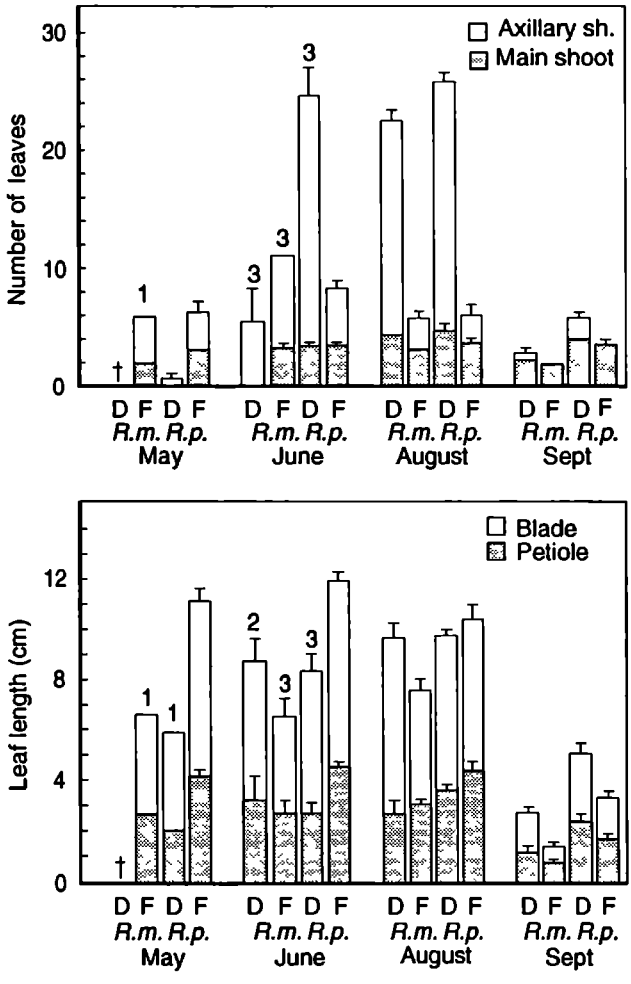
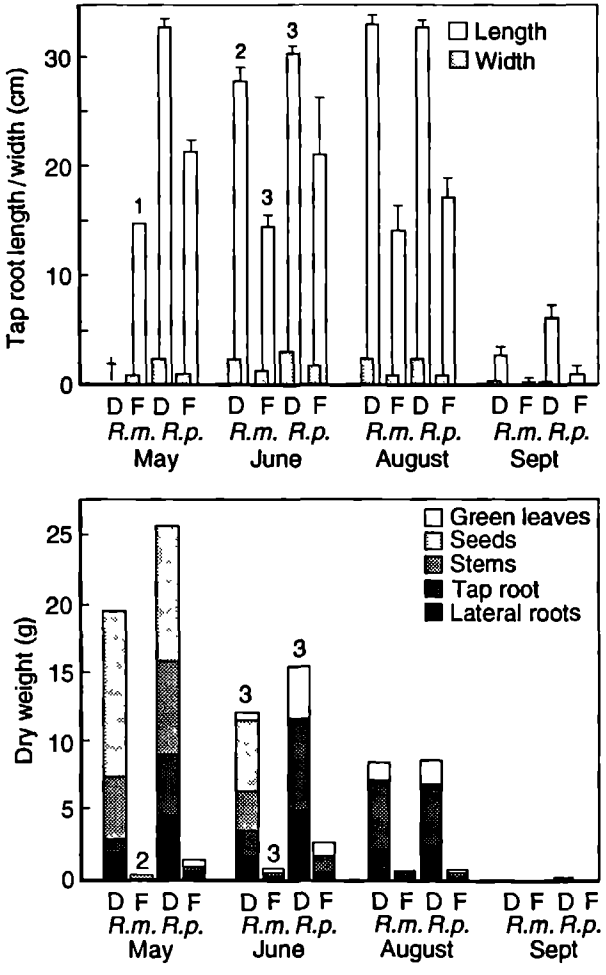


Fig. 1. Mean plant parameters (±1SE, n=4 or indicated if less) at the end of the first growing season (December 1987) of plants of *Rumex maritimus* and *Rumex palustris* from different cohorts and flooding treatments (D: drained; F: flooded). Tap root length: length of the part with a diameter > 1 mm; Tap root width: maximum diameter at the root-shoot junction. † All plants died after flowering of main and axillary shoots in 1987 (see Chapter 4 for details of this experiment).

1989 these plants had all received the same treatment: drained until January 1989 and flooded from 9 January up to 10 April 1989). In 1988, seed mixtures from five plants of *R. maritimus* from the same August cohort of 1987 could be used. In 1989, five plants per treatment from the July cohort of 1988 were taken. The mean fifty-seed weight was determined twice (because of the small sample size) of sixteen samples per treatment. Germination characteristics were studied under conditions of constant and fluctuating temperatures as described in Chapter 4.

Statistical analyses were performed with the SAS statistical package (SAS Institute Inc. 1985) using the General Linear Models procedure for analysis of variance with unequal cell sizes and Bonferroni t-tests to compare separate means.



Results

Field observations

In 1976, the early exposure of mud flats allowed abundant flowering in *R. maritimus* in the year of germination (Table 1). Early in the following season, flowering plants of *R. palustris* were frequently observed, but none of *R. maritimus*. It appears from Table 1 that annuality is largely restricted to *R. maritimus* and only under rather exceptional conditions of early subsidence of floods and shallow flooding in the first growing season. Flowering and fructification are frequently observed in the second growing season in both species if not prohibited by severe flooding. During field observations in December 1987, large numbers of small plants of *R.*

palustris were observed with two-three leaves up to 5 cm in length. These appeared to be vegetative shoots sprouting from the axils of stem leaves of large flowering plants which had fallen over as a result of prolonged flooding in this year. These flowering plants could not have germinated in 1987 due to the late exposure in this year (Table 1).

Extremely dry conditions during the growing season of 1989 resulted in high mortality of plants of both species which had germinated in 1988 and had survived the following winter. Survivors flowered in 1989 but had a very low seed output (Table 1), probably due to drought stress and heavy predation by the chrysomelid beetle *Gastrophysa viridula.*

Table 1: Life histories in *Rumex maritimus* and *Rumex palustris* in relation to flooding conditions over the periods 1976-80 and 1987-89 on a river-bed shore in the Kekerdomse waard near Nijmegen (soil level 9.30-9.80 m +NAP=Amsterdam Ordnance Datum). Data from field observations (1976 and 1987-89) and relevés (1977-80).

Year of germination	Time of exposure *a*	First growing season			Second growing season *c*		
		Flooding conditions	Fructification *b*		Flooding conditions	Fructification *b*	
			R. m.	*R. p.*		*R. m.*	*R. p.*
1976	April	None	++	-	Short, shallow	-	+
1977	June-September	Short, shallow	-	-	Long, deep	(+)	(+)
1978	April-August	Long, deep	-	-	Short, shallow	+	+
1979	July	Short, shallow	-	-	Long, very deep	-	-
1987	September	Very long, deep	-	-	Short, shallow	- *	- *
1988	June-July	Short, shallow	+	(+)	None	(+)	(+)

a Theoretical soil exposure with respect to river-water level for at least 7 successive days from April onwards. Real exposure may have occurred later in the period 1976-79 (see text); *b* Fructification: ++ abundant, + frequent, (+) sparse, - none. Germination and establishment occurred in all seasons; *c* After a winter period with at least 4 months of flooding with a maximum depth of over 4 m above the soil; *All plants were winter-killed.

Outdoor flooding experiments

During the winter months, drained plants grew very slowly with approximately one new leaf per month and an equal death rate. Leaves which developed during the winter were darker green, smaller, and had less curly margins than summer leaves, which appeared from mid April onwards.

Notwithstanding the low water temperatures, that remained below 5 °C up to early March 1988, under-water leaf elongation was observed in the flooded plants from the onset of winter flooding onwards. In the course of February, plants of *R. maritimus* from the August cohort of 1987 started to emerge from the water surface, followed by plants from several cohorts of *R. palustris* (Fig. 2). Several weeks later, bolting was observed in emergent plants. Adventitious roots sprouted first from the basal stem node of these plants and later also from higher stem nodes (approximate time indicated in Fig. 2). In the last week of flooding, maximum water temperatures were near 20 °C (17.00 h).

Four months of winter flooding caused the death of some of the smallest plants (September cohort of 1987) and of some plants which had only axillary shoots left after flowering of the main shoot in 1987 (Table 2). Maximum leaf, petiole and stem lengths at the end of the flooding period are presented in Fig. 3. Plants of both species tended to raise leaf blades above the water surface and relative petiole length was largest in plants which still possessed relatively short stems. Leaf numbers of main and axillary shoots showed differences between the species (Table 3). The number of axillary leaves was more reduced in *R. maritimus* than in *R. palustris*. Especially plants of *R. maritimus* which had received at least one of both flooding treatments (summer 1987 or winter 1988), either invested in main or in axillary shoots.

Table 2: Percentage survival (*n* between brackets) in *Rumex* spp. after different winter treatments (flooded from 9 January-10 May 1988, water-level 40 cm above the soil, or drained throughout this period). Plants from different cohorts and summer flooding treatments of 1987; mean plant size at the onset of the winter is presented in Fig. 1.

Summer treatment:	Drained	Drained	Flooded	Flooded
Winter treatment:	Drained	Flooded	Drained	Flooded
Cohort of 1987, *R. maritimus*				
May	-(0)	-(0)	100(1)	0(2)
June	100(3)*	100(3)*	89(7)**	67(6)**
August	100(8)	100(8)	100(8)	100(8)
September	100(8)	88(8)	100(8)	75(8)
Cohort of 1987, *R. palustris*				
May	100(4)*	50(4)**	100(8)	100(8)
June	100(8)	100(8)	100(8)	100(8)
August	100(8)	100(8)	100(8)	100(8)
September	100(8)	88(8)	100(8)	63(8)

*Plants with only vegetative axillary shoots left after flowering of the main shoot in 1987; **Plants with either main and/or axillary shoots (part of these plants had flowered in 1987).

All plants of 1987 reproduced in the growing season of 1988. Inflorescences appeared from half May onwards in the case of winter-flooded plants and from the end of May onwards in winter-drained plants of both species. Seed ripening was completed at the end of July in most plants with minor differences between species and between summer and winter treatments. However, plants which emerged from the water surface in March were among the first to produce inflorescences. Plants from the September cohort of 1987 only started to flower in July and ripened at the end of August. Compared with annual plants, the reproductive development of biennial plants of both species took place much earlier in the growing season (see Chapters 3, 4 and 5).

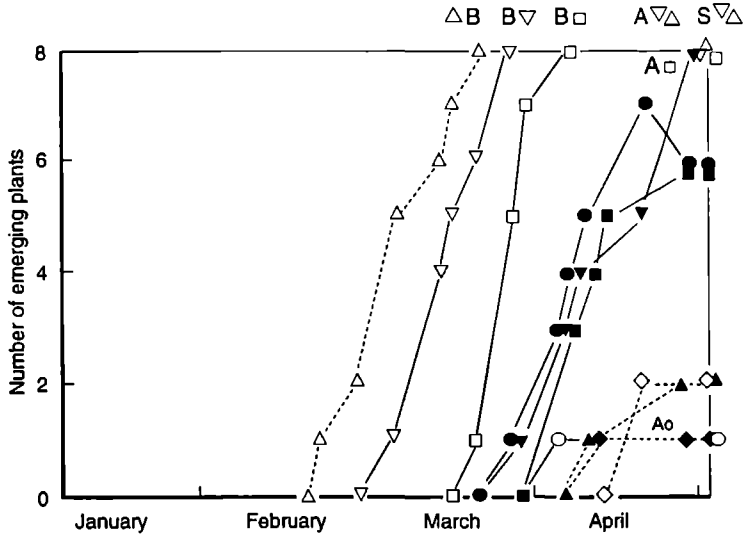


Fig. 2: Emergence from the water surface during 4 months of winter flooding (40 cm above the soil) in *Rumex maritimus* (June cohort: △ drained and ▲ flooded; August cohort: ◇ drained and ◆ flooded) and *Rumex palustris* (May cohort: ○ drained and ● flooded; June cohort: ▽ drained and ▼ flooded; August cohort: □ drained and ■ flooded). The approximate times of: (B) bolting, (A) adventitious rooting, and (S) emergence of reproductive stems are indicated per cohort and species. See Fig. 1 for further details.

Table 3: Number of living leaves present on main and axillary shoots (mean±1SE, *n* between brackets) in *Rumex* spp. immediately after winter flooding in May 1988. See Figs. 1 and 2 for further details.

Summer:	Drained		Drained		Flooded		Flooded	
Winter:	Drained		Flooded		Drained		Flooded	
	Main sh.	Axill. sh.	Main sh.	Axill. sh.	Main sh.	Axill. sh.	Main sh.	Axill. sh.
Cohort of 1987, *R. maritimus*								
May	-	-	-	-	4.0(1)	-	-	-
June	-	5.7±1.3(3)	-	4.3±1.2(3)	3.7±0.3(3)*a*	6.4±1.8(5)*a*	2.0±0.0(2)*b*	11.0(1)*b*
August	6.1±0.2(8)	8.1±1.1(8)	6.4±0.3(8)	-	4.0±0.3(8)	-	2.0±0.3(8)	-
Sept.	3.6±0.3(8)	-	1.4±0.3(7)	-	2.4±0.4(8)	-	1.2±0.3(6)	-
Cohort of 1987, *R. palustris*								
May	-	19.5±4.5(4)	4.0(1)*c*	9.0±6.0(2)*c*	4.5±0.2(8)	2.0(1)	2.6±0.3(8)	-
June	4.1±0.2(8)	17.4±1.2(8)	4.0±0.3(8)	4.0±1.0(3)	4.4±0.2(8)	4.3±0.5(7)	2.9±0.4(8)	-
August	4.9±0.1(8)	16.5±0.6(8)	5.8±0.3(8)	1.0(1)	4.5±0.3(8)	1.8±0.5(4)	2.6±0.6(8)	-
Sept.	3.1±0.1(8)	-	0.2±0.2(5)	-	3.0±0.0(8)	-	0.8±0.5(5)	-

a Total *n*=6; *b* Total *n*=3; *c* Total *n*=2.

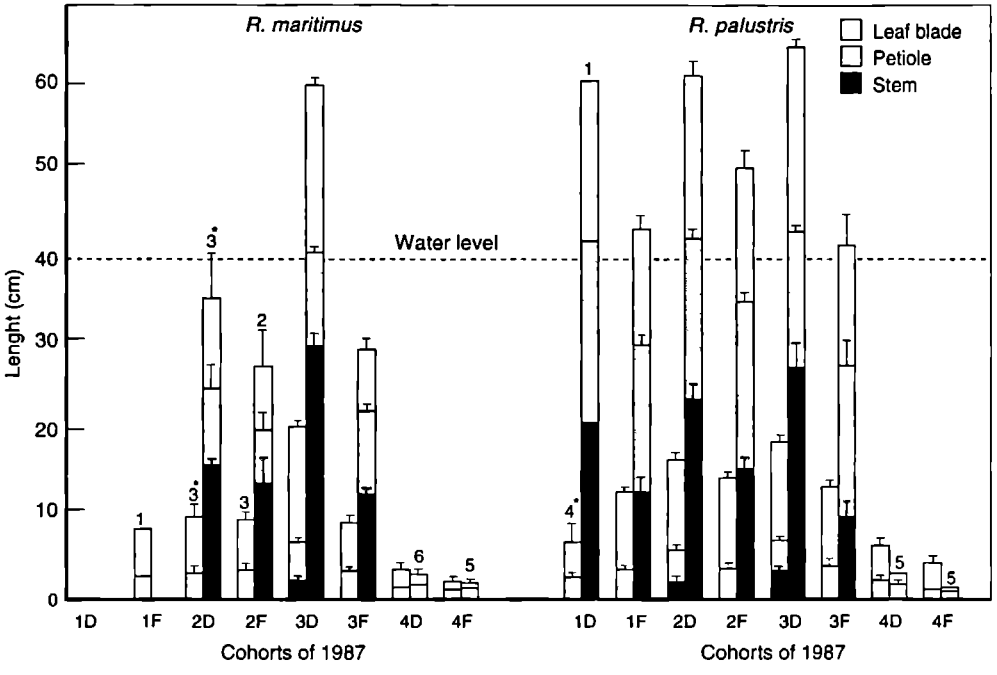


Fig. 3: Approximate plant heigth (mean maximum leaf and stem lengths ±1SE, *n*=8 or indicated if less; *axillary shoot, else main shoots) per cohort of 1987 (1: May; 2: June; 3: August; 4: September) and summer treatment (D: Drained; F: Flooded) in *Rumex maritimus* and *Rumex palustris* immediately after 4 months of winter flooding (water level indicated). Each pair of observations represents the winter-drained (first bar) and the winter-flooded (second bar) plants respectively. See Fig. 1 for further details.

Thirty-four % of all plants of *R. palustris* survived after flowering in 1988 with vegetative axillary shoots. Plants from all treatments and cohorts of 1987, except the September cohort, were represented, including some plants from the May cohort which had flowered both in 1987 and 1988.

Shoot emergence during the following winter flooding period (1989) occurred earlier and more frequent in *R. palustris* in the cohorts of 1988 (100% in the July and 50% in the June cohort, respectively) compared with *R. maritimus* (50% and 44%, respectively), but all plants survived. In the thirty-eight plants of *R. palustris* from 1987, 20% emerged. These were all plants from the June and August cohorts which had not been flooded up to the second winter flooding period. Of those which remained submerged, six plants died.

At the onset of the early summer flooding treatment in 1989, inflorescences were present in all plants of both species. However, all plants from 1987 and part of the plants from 1988 (mostly of *R. maritimus*) were completely submerged at the onset of flooding. Of those which remained

submerged, approximately 50% died. After the flooding period in 1989, most plants were out of flower with minor differences between drained and flooded plants.

Seed production in 1988 (Table 4) showed differences per cohort and summer flooding treatment as expected from differences in biomass at the onset of the winter (Fig. 1). Surprisingly, however, the seed output of winter-flooded plants was larger than of non-winter-flooded plants in cohorts in which plants emerged during the flooding period. In these cohorts, plants which reached the water surface produced significantly more seeds than plants which were unable to do this (data not shown). In 1989, the seed output of summer-flooded plants and drained plants of either 1987 or 1988 was not significantly different in both species (data not shown). However, in the plants of *R. palustris* from 1987 less than 2 g seeds were formed by the five plants which did not emerge during the summer flooding treatment compared to *c.* 5 g in the ten emergent plants.

Irrespective of earlier flooding conditions, plants of both *Rumex* spp. produced relatively large seeds in the second and third growing seasons (Table 5) compared with annual plants (Chapters 3-5). Germination characteristics of seeds from biennial or triennial plants were not different from seeds of annual plants (data not shown).

Table 4: Mean seed production in g/plant (*n* between brackets) in *Rumex* spp. in the second growing season (1988) after 4 months of winter flooding (40 cm above the soil) in relation to summer flooding treatments given in the first growing season (1987). See Fig. 1 for further details.

Summer treatment:	Drained	Drained	Flooded	Flooded
Winter treatment:	Drained	Flooded	Drained	Flooded
Cohort of 1987, *R. maritimus*				
May	-	-	0.72(1)	-
June	0.42(3)**c*	2.45(3)**a*	0.48(5)*c*	1.75(4)*b*
August	5.14(8)*b*	6.58(8)*a*	0.80(8)*c*	1.34(7)*c*
September	0.35(8)*a*	0.31(6)*a*	0.11(7)*b*	0.19(4)*ab*
Cohort of 1987, *R. palustris*				
May	0.92(4)**b*	-	1.38(1)*b*	3.35(8)*a*
June	7.91(8)*a*	7.40(8)*a*	2.42(8)*c*	3.48(8)*b*
August	6.99(8)*b*	9.49(8)*a*	1.50(8)*c*	1.68(8)*c*
September	0.52(8)*a*	0.71(5)*a*	0.34(8)*a*	0.31(5)*a*

*Seeds produced by axillary shoots of plants which had flowered with the main shoot in 1987. Means followed by the same letter (within-cohort comparisons) are not significantly different (Bonferroni *t*-test, $p<0.05$).

Table 5: Mean 50-seed weight in mg (±1SE, n=22) of biennial and triennial plants of *Rumex* spp. in relation to earlier flooding treatments (see text).

Summer 1987(88*)	Winter 1988(89*)	Summer 1989	*R. maritimus*	*R. palustris*
Drained	Drained		11.0±0.1*b*	30.9±0.2*b*
Drained	Flooded		12.7±0.2*a*	29.4±0.2*c*
Flooded	Drained		12.7±0.3*a*	31.4±0.2*b*
Flooded	Flooded		12.9±0.3*a*	31.4±0.2*b*
(Drained*)	(Flooded*)	Drained	10.1±0.4*c*	30.6±0.2*b*
(Drained*)	(Flooded*)	Flooded	10.4±0.2*c*	33.0±0.2*a*

*Seed output in 1989: plants of *R. palustris* from the August cohort of 1987 and plants of *R. maritimus* from the July cohort of 1988. Other data: plants of both spp. from the August cohort of 1987. Means followed by the same letter are not significantly different (within-species comparison, Bonferroni t-test, p<0.05).

Discussion

Although *R. maritimus* and *R. palustris* are both capable of flowering in the first growing season (*e.g.* Chapter 3), this is not commonly observed in river forelands (Table 1). In *R. palustris*, the annual strategy seems to be even less important than in *R. maritimus*. In *R. palustris*, next to biennality the short-lived perennial strategy was observed in outdoor experiments. Field observations and studies of these species in other wetland systems (Hejny 1960) also indicated the occurrence of vegetative proliferation in *R. palustris*. Axillary shoots initiated from nodes of collapsed flowering stems may root and become independent of the primary shoot. By this means, plants of *R. palustris* escape from the structural constraints of large flowering stems and thus profit to some extent from the advantages of clonal growth (see Watkinson & White 1987).

Studies on flooding in the winter season in experimental plots (Table 2) suggest that in both species survival of winter flooding is lowest in the lowest areas of mud flats in river forelands due to late germination as well as more severe flooding during the first growing season, which both result in plants of a small mean size at the onset of the winter. In the highest areas of mud flats, survival may be relatively low as well after flowering in the first growing season, which also reduces the mean size of plants at the end of the season. Differences in tolerance of winter flooding between both species were observed as differences in the survival of axillary shoots (Table 3). A higher flooding tolerance in *R. palustris* compared with *R. maritimus* (see also Chapters 4 and 5) is clearly coupled to a longer life history. These conclusions are supported by other field observations (A.J.M. van der Sman, unpublished). As a consequence of the higher incidence of flowering in *R. maritimus*, this species is more likely to behave as a monocarpic species in river forelands than *R. palustris*. Only the ramets should be considered as monocarpic in the latter species (Harper 1977; Watkinson & White 1985). For this reason, *R. palustris* cannot be nicely fitted in the categories suggested by Klinkhamer, de Jong & Meelis (1987) with respect to the control of flowering in short-lived species.

Shoot emergence was important for the survival of prolonged winter flooding and early-summer flooding in the following season. However, survival of several months of complete submergence in winter and spring was much higher than of only 4 weeks of summer flooding in both species (see also Chapter 5). To some extent, this can be explained by relatively low water temperatures and consequently low metabolic rates in winter. The presence of typical small, dark-green winter leaves in both species, which may have been induced by short photoperiods as observed in grasses (Hay 1990), indicates a state of dormancy. Dormancy during harsh seasons may be an important additional strategy in the survival of wetland plants (see Hook 1984; Braendle & Crawford 1987).

Under-water stem elongation in early spring apparently initiated reproductive development, and emergence enhanced seed production in the following season. All plants of both species reproduced relatively early in following seasons compared with the first growing season (Chapters 3 and 4). This may be of advantage in unpredictable environments (Hook 1984). Since even the smallest plants from the September cohort of 1987 had initiated more than twelve main leaves in the period between the subsidence of winter flooding and the onset of bolting in June 1988, the control of flowering seems to depend on stage rather than size in the second growing season as well as in the first season in these species (see also Chapters 3 and 4).

Seed output in the first and second growing seasons may be compared in the summer-flooded plants of *R. maritimus* from the June cohort of 1987. Several of these plants remained vegetative in 1987 while larger plants flowered (Chapter 4). Based on their estimated biomass (number of living leaves x maximum leaf length, see Chapter 2) the non-flowering plants would have had a mean estimated seed output of 0.15 g in 1987. Mean seed weight, including perianths, was 0.216 mg in 1987 which results in an estimated seed number of 714 per plant. The real mean seed output of these plants in 1988 was 1.61 g. Mean seed weight, including perianths, was 0.516 mg resulting in 3120 seeds per plant. The seeds produced by plants in 1988 were not only twice as heavy as those from plants which reproduced in 1987, but also did not show reduced dormancy levels as in the previous year (Chapter 4). We need to know much more on the survival of rosettes under field conditions as well as on seed survival which is probably much lower in low-quality seeds, before to decide which strategy is most advantageous in *R. maritimus*. The chances of seeds from annual and biennial plants to produce more seeds in following growing seasons may differ due to differences in seed size in *R. palustris* as well (Table 5).

The relative advantage of different life-history strategies in both species is more clear. Biennial plants of *R. palustris* had a higher seed output in the second season than those of *R. maritimus* (Table 4) and several reproduced once more in the third season. In contrast, annual plants of *R. palustris* under a number of flooding regimes had a lower seed output than those of *R. maritimus* (Chapters 3-5). As a result, *R. palustris* produced much more seeds after the first growing season under varying conditions of flooding compared with *R. maritimus*.

Field studies may raise unexpected problems in unpredictable environments. The first year of observations in the period 1987-89 provided an extremely wet season which resulted in the death of all established plants of both species in the following winter (Table 1). Plants which germinated in the following season and survived winter flooding were confronted with conditions of extreme drought in 1989. The outbreak of *Gastrophysa viridula* in this season may have been caused by drought stress after a wet winter (see White 1974). These observations reveal that extremely fluctuating water levels in river forelands do not only result in disturbance and stress due to flooding, but also in drought stress. In the absence of disturbance and stress

due to water-level fluctuations, competition may become more important in the dense stands which are formed by these species (see Tilman 1988). The field situation is extremely complex and it may even seem rather surprising that our results from experimental plots match so well with field observations.

Summarizing the results according to the three tactics of stress resistance suggested by Fitter & Hay (1981), we found the tactic of avoidance of flooding to be more important in *R. maritimus* and the tactic of flooding tolerance to be more important in *R. palustris* (see also Hejny 1960). Shoot elongation and subsequent adventitious rooting represent the intermediate tactic of amelioration which is observed in both species. Its relative importance for the survival of flooding and reproduction afterwards is probably highest in the first growing season in the avoidant species *R. maritimus* and in the second and third growing seasons in the tolerant species *R. palustris*. These differences in tactics are reflected in differences between the life histories of both species.

Acknowledgments - We thank Nina Joosten, Christel Vetters and Miranda Slegers for help with the experiments.

THE ROLE OF ETHYLENE IN SHOOT ELONGATION WITH RESPECT TO SURVIVAL AND SEED OUTPUT OF FLOODED RUMEX MARITIMUS PLANTS

with L.A.C.J. Voesenek, C.W.P.M. Blom, F.J.M. Harren and J. Reuss

Rumex maritimus occurs in frequently flooded sites of lowland floodplains. Upon submergence this species exhibited rapid elongation of shoots, but the response depended on the developmental stage when flooding was initiated. In the rosette stage, petioles showed a fast and large response; during early stem elongation the response of petioles was less, but the extension of the lower internodes considerable; during flower-stem development, higher internodes extended and the contribution to final stem length diminished. The elongation responses could in part be mimicked by exogenous ethylene.

Ethylene production by an intact plant before, during and after submergence was measured with a laser-driven photoacoustic technique. Internal ethylene concentrations increased within twelve hours of submergence. Ethylene accumulated in the submerged plant due to increased synthesis as well as a reduced diffusion from the plant to the water (instead of air). Immediately after submergence a further increase in ethylene synthesis was observed, which may be of vital importance in causing shoots to continue their rapid elongation even after the water surface is reached.

The responses of shoots were related to fitness in experimental field plots. Survival of submerged plants of *R. maritimus* depended on their ability to emerge from the water surface, while seed output was positively correlated with shoot height above the water.

Introduction

Downstream river systems in The Netherlands are frequently flooded, not only during winter high-waters, but in the growing season as well (Van de Steeg 1984; Brock, Van der Velde & Van de Steeg 1987). Plant performance in these river areas depends upon mechanisms to survive and reproduce during fluctuating water levels, including periods of complete submergence (Blom *et al.* 1990). Several *Rumex* species are distributed across the river floodplain according to the elevational gradient. *Rumex maritimus* L. occurs in the most frequently flooded areas and plants of this species may encounter flooding in several stages of their life cycle.

Flooding imposes a severe stress on plants. Complete submergence of the shoot will result in reduced aeration of the plant tissues and an impaired photosynthesis (Ridge 1987). Survival and sexual reproduction may depend on adaptations which restore contact between shoot and air. A commonly observed mechanism is the rapid elongation response of petioles and/or internodes, which is stimulated by ethylene accumulation following submergence. The occurrence of this so-called "depth accommodation" and the role of ethylene in the process have been studied for amphibious plants since 1970 (reviewed by Osborne 1984; Jackson 1985; Ridge 1987) and have been recently demonstrated for several *Rumex* species (Voesenek & Blom 1989a,b; Voesenek *et al.* 1990a). Not many studies have been undertaken to assess the impact of this response on fitness. Ridge (1987) discussed the survival value of several morphological and anatomical changes induced by flooding, including elongation responses. The importance of shoot

elongation for the survival of *Rumex* spp. was demonstrated by Voesenek (1990) and Laan (1990).

In this study we investigated the impact of rapid shoot elongation on the fitness of flooded plants of *R. maritimus*. An outdoor experiment was designed to address this question in plants at different stages of the life-cycle. Survival and seed production were used to determine fitness. To elucidate the mechanism behind the observed rapid elongation growth, the role of ethylene was studied in several laboratory experiments. To monitor production and accumulation of ethylene in submerged plants we used the recently developed laser-driven photoacoustic detection technique (Harren 1988; Harren *et al.* 1990) that has been used previously for the measurement of ethylene production of waterlogged *Rumex* plants (Voesenek 1990; Voesenek *et al.* 1990b).

Materials and methods

Plant material

Rumex maritimus is a tap-rooted rosette species with petiolate leaves, annual in a 16-h or longer photoperiod, but otherwise biennial (see Chapters 2-6). The reproductive stage starts with the elongation of internodes (bolting); the first stretched internode appears between the sixth and seventh rosette leaf or even later, depending on external conditions. Under favourable conditions axillary shoots are initiated in all main rosette and stem leaves. The shoots appearing from the rosette leaf axils can also elongate, producing axillary stems. These stems may be induced together with the main stem or in the following growing season. Plants grown in small (6-cm) pots in the greenhouse do not initiate axillary shoots.

Seeds of *Rumex maritimus* were harvested in 1986 in the Kekerdomse Waard near Nijmegen. A mixture of seeds from five plants was preserved dry in the dark at room temperature until use. Germination was always achieved as follows: seeds without perianths were placed in petri dishes (diameter 90 mm) on two layers of filter paper (Schleicher and Schüll 595), moistened with demineralized water. The petri dishes were placed in a germination chamber under an alternating temperature and light regime (12 h light 25 °C/ 12 h dark 10 °C; mean photon flux density [PAR: 400-700nm]: 30 µmol m^{-2} s^{-1}). As soon as radicles emerged, after approximately 4 days, seeds were planted in pots, filled with a 1:1 mixture (v:v) of a potting compost (Jongkind, No.5) and river sand. Several seeds were planted per pot; eventually seedlings were thinned to one plant per pot.

Outdoor experiment

This experiment was conducted in 1988 in large basins (length x width x depth: 8 x 2.4 x 1 m). Plants were grown in PVC pots (height 50 cm, diameter 16 cm). Four cohorts of plants of *R. maritimus*, twenty-four per cohort, were grown under drained conditions (*i.e.*, the pots stood in the basins in approximately 5 cm of water). The first cohort was planted on 2 May 1988; the following cohort 2 weeks later and so on, until the last cohort was planted on 13 June. After 11 weeks from the start of the experiment (18 July) a flooding period of 4 weeks was simulated by filling one of the basins, containing twelve plants from each cohort, with tap-water. Submergence depth was 40 cm above the brim of the pots. After 4 weeks the water was pumped out of the basin. Filling as well as emptying the basin took approximately 8 h. Plant heights

were measured before and after the flooding treatment and at the end of the growing season; generative development was recorded throughout the experiment. After the flooding period, the length of the internodes of main stems and of the first three axillary stems were measured per plant. At the end of the growing season, ripe seeds, including perianths, were harvested and the seed output of each plant was determined.

Mean daily temperatures were recorded throughout the experiment. These fluctuated around 16 °C during the first 6 weeks of the experiment and thereafter gradually rose to 18 °C at the end of the flooding period (15 August). Water temperatures ranged from 14/19 °C (09.00 h/17.00 h) during the first week of flooding up to 20/26 °C during the last week of flooding. Statistical analyses on data were performed with the General Linear Models (GLM) procedure of the SAS statistical package (SAS Institute Inc. 1985).

Ethylene experiments

All greenhouse and laboratory experiments were conducted in the period from 1988 to 1990 with plants in small pots (height 6 cm; diameter 5.5 cm). The experiments with exogenous ethylene were performed in a growth chamber (PAR: 140 µmol m^{-2} s^{-1}, Philips TL8W/33XE3 tubes). The plants used for the measurements of internal ethylene concentrations and for the photoacoustic measurements of ethylene production were grown in the greenhouse (PAR at least 110 µmol $m^{-2}s^{-1}$ from the additional light source: high pressure sodium lamps, 400 W, Philips HPS/SonT). The photoperiod was 16 h in all cases. Temperature was kept constant at 20 °C in the growth chamber. In the computer-controlled greenhouse, temperatures fluctuated between 20 °C (daytime) and 16 °C (night). Experiments were performed with plants of *R. maritimus* in three stages: the rosette stage with five leaves larger than 1 cm in length present, the bolting stage with eight leaves and three internodes larger than 1 cm, and the early flowering stage. These stages were reached after approximately 5, 7 and 11 weeks, respectively.

The effect of externally applied ethylene was studied in a desiccator (300 mm diameter) containing eight plants. The desiccator was daily flushed with an ethylene/air gas mixture (ethylene concentration $5x10^{-6}$ m^3m^{-3}) for 4 days. Daily flushing was proven sufficient to maintain a nearly constant ethylene concentration (Voesenek & Blom 1989b). Results were compared with eight plants submerged in tap-water in a glass container and another eight plants in a desiccator flushed with medical air (ethylene concentration $4x10^{-9}$ m^3m^{-3}) to serve as the control. This experiment was carried out twice with essentially similar results; for each developmental stage the results of only one experiment are presented.

To determine endogenous ethylene levels we used the vacuum extraction method of Beyer & Morgan (1970) to extract gases from the plant material (see also Voesenek & Blom 1989b). For each 10^{-6} m^3 gas sample we used excised leaf, petiole and internode parts of 3-4 cm length from three or four plants. Ethylene concentrations in these samples were measured with a Chrompack Packard gas chromatograph at 60 °C oven temperature, using a packed Poropack Q column (100 cm) filled at a density of 0.34 g cm^{-3}. All measurements were repeated three or four times in the rosette and bolting stages after 12, 24 and 36 h of submergence and in the flowering stage only after 12 h of submergence. Results were compared with internal ethylene concentrations of drained plants.

Ethylene entrapment in and production of an intact plant before, during and after a 24-h period of submergence was measured with the recently developed laser-driven photoacoustic detection system (Harren 1988) in combination with a flow-through system. This apparatus allows monitoring of ethylene in a continuous air flow along the plant tissue, thereby avoiding

autocatalytical or autoinhibitory processes as well as possible interactions of ethylene with varying concentrations of O_2 and CO_2 (Woltering, Harren & Boerrigter 1988). This highly sensitive method has been refined (Hess 1989; Harren *et al.* 1990) allowing measurement of ethylene concentrations in air as low as $6x10^{-12}$ m^3m^{-3} Since the method for the measurement of the ethylene production by waterlogged plants of *Rumex* is described in detail elsewhere (Voesenek 1990; Voesenek *et al.* 1990b) we will only mention the underlying principles here.

The photoacoustic effect is based on the generation of acoustic waves due to the accumulation of heat in a sample. In our case the sample is a volume of ethylene/air gas and the ethylene molecules are excitated by a powerful CO_2-laser beam. Excitation is followed by relaxation, resulting in an increase in temperature which in turn leads to a corresponding increase in pressure inside the resonator of the photoacoustic cell. These pressure changes can be detected by a microphone and the magnitude of the signal is proportional to the number of absorbing molecules. The photoacoustic equipment was used in connection with a flow-through system, consisting of three separate cuvettes ($3x10^{-4}$ m^3), each containing an intact plant. PAR at plant level was a constant 50 µmol $m^{-2}s^{-1}$. A continuous air flow (flow rate: $3x10^{-3}$ m^3h^{-1}) from which all carbohydrates were removed was conducted through each cuvette. After passing the plant tissue, the air was conducted to a glass column filled with KOH grains to remove water and CO_2 and thereafter through the photoacoustic cell. Cuvettes were either connected with the photoacoustic cell, or the effluent was vented into the surrounding air.

Two different experiments were performed with plants of *R. maritimus* in the stem elongation phase. In the first experiment, three plants were kept drained in the cuvettes during an acclimatization period of at least 12 h. Thereafter one plant remained in drained condition as control. The second plant was submerged for 24 h. During this time ethylene was measured in the air space above the water surface. After 24 h the water level was lowered to waterlogging conditions (1 cm above soil) to be able to measure the amount of accumulated ethylene. We prevented the infiltration of air from outside the system into the cuvettes in order not to disturb the measurement. The production level of this plant was compared with the production level of a third, waterlogged plant. The three cuvettes were measured in turn (five measurements per cuvette in approximately 30 min) until the end of the submergence period. After lowering the water level, only the cuvette containing the previously submerged plant was measured for a period of 12 h. Over the last 100 h, the three cuvettes were measured in turn. This experiment was repeated three times with essentially similar results. The results of only one experiment are presented. Separate measurements were taken to ensure that the production level of the waterlogged plant did not change during the 12 h when it was not measured in the former experiments and to assess the mean production level of drained plants, using an empty cuvette as a reference for zero ethylene production. In the second experiment, the submergence treatment was repeated but this time in comparison with submergence in AVG (amino-ethoxyvinylglycine)(0.1 mol m^{-3}), an inhibitor of ACC(1-aminocyclopropane-1-carboxylic acid)-synthesis, to prevent the production of ethylene.

Results

Outdoor experiment

At the start of the flooding period the first three cohorts of plants of *R. maritimus* had reached the bolting stage; the first was already flowering (mean primary stem length 46 cm), the second

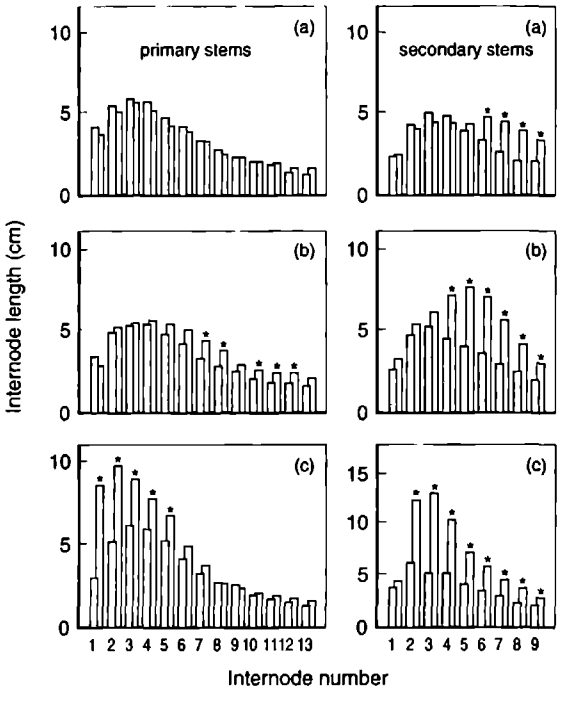


Fig.1. Mean internode lengths of main and axillary stems (sprouting from the axils of the first three rosette leaves) in *Rumex maritimus* at the end of the outdoor flooding experiment. Open bars: drained; shaded bars: flooded for 4 weeks (40 cm above the soil) at the age of (a) 11 weeks, (b) 9 weeks, (c) 7 weeks (n=12). Internodes are numbered in the order of appearance (the first stretched internode of the main stem appeared between rosette leaves 7 and 8). *Significantly different (p<0.05).

about to flower (mean primary stem length 37 cm), and the third was in the early bolting stage (mean primary stem length 10 cm). Only two plants from the first cohort were completely submerged, and these remained under water and died during the flooding treatment. The second and third cohorts of plants were completely submerged, but reached the water surface within a week. All plants which emerged from the water, although some with only a very small (<9 cm) top part of the primary stem, survived and were flowering by the end of the flooding period. The lag in development of flowers between drained and flooded plants ranged from 1 day (first cohort) up to 10 days (third cohort). The fourth cohort was still vegetative at the start of the flooding treatment. These plants possessed approximately eight main leaves with a maximum leaf length of 10 cm. During submergence, leaves quickly elongated up to approximately 30 cm, but not one of the plants was able to reach the water surface and all died within 2 weeks.

At the end of the growing season, drained plants from all cohorts had attained equal stem lengths (Table 1). No additional main-stem extension due to flooding was measured in the already flowering plants from the first cohort. The largest stems were observed in the plants from the third cohort which had been flooded in the early bolting stage. This was correlated with the increased length of the first five main-stem internodes in these plants, immediately after flooding (Fig. 1). Further developed stems showed increasingly smaller extension of higher internodes upon flooding. In the first cohort the elongation response was only significant for the upper internodes of axillary stems, which in development always lagged behind main stemstems by approximately 10 days.

Table 1. Mean length in cm (±1SE) of main stems in *Rumex maritimus* at the end of the growing season in the outdoor experiment; the flooding treatment (water height 40 cm above soil level) of 4 weeks was started at the age of 11 (cohort I), 9 (II), 7 (III) and 5 (IV) weeks, with 12 plants per cohort and treatment.

	Drained	Flooded
Cohort I	52.2±2.5*a*	52.3±2.0*a*
Cohort II	51.8±2.0*a*	59.5±3.5*b*
Cohort III	53.4±2.0*a*	73.3±2.0*c*
Cohort IV	51.5±3.4*a*	-*

*All flooded plants died during the flooding period. Means followed by the same letter are not significantly different ($p<0.05$).

Table 2. Mean seed production in g/plant (±1SE, $n=12$) per cohort and treatment (see Table 1 for details).

	Drained	Flooded
Cohort I	18.7±0.6*b*	8.1±1.0*a*
Cohort II	19.4±0.3*b*	9.2±1.7*a*
Cohort III	19.0±0.8*b*	10.1±0.8*a*
Cohort IV	10.4±0.7*a*	-

Means followed by the same letter are not significantly different ($p<0.05$).

Table 3. Mean seed production in g/plant (±1SE, *n* between brackets) per length class (main stem length in cm) in *Rumex maritimus* (see Table 1 for details).

Length class	Cohort I		Cohort II		Cohort III	
	Drained	Flooded	Drained	Flooded	Drained	Flooded
<50	18.6±1.5(4)*a*	3.7±0.2(3)*e*	18.9±0.3(5)*a*	2.8±1.1(4)*e*	17.6±0.9(4)*a*	-
50-54	18.3±1.2(4)*a*	7.3±0.4(4)*d*	19.4±0.5(3)*a*	6.3(1)	18.6±2.1(3)*a*	-
>54	19.2±0.2(4)*a*	11.4±1.0(5)*bc*	20.0±0.7(4)*a*	13.7±0.7(7)*b*	18.9±1.6(5)*a*	10.1±0.8(12)*c*

Means followed by the same letter are not significantly different ($p<0.05$).

All flowering plants produced at least some viable seeds (even the two plants from the first cohort which were dead after the submergence period). There were no differences in seed output between the first three cohorts of drained plants (Table 2). The fourth cohort produced only half the amount of the earlier cohorts. Seed production was reduced in flooded plants compared with drained plants. Differences between flooded cohorts were not significant, but within each cohort a large variation existed which was associated with variation in stem length (Table 3). A very low seed production was recorded for plants which had exhibited very little or no extension growth and, as a consequence, remained submerged or appeared for less than 9 cm above the water surface (length class <50 cm). An intermediate value was reached by plants in the category ranging from 10-14 cm above water level (50-54 cm). Most seeds were produced

by plants which were larger than 54 cm. Drained plants from all three cohorts were equally divided over these three length classes and showed no correlation between shoot height and seed production. Most of the flooded plants in the third length class, especially those from the third cohort, were much larger (up to 89 cm) than drained plants, which did not exceed 64 cm. The largest plants were always the ones with the least developed stems at the start of the flooding treatment (yet with the stem already initiated), which exhibited the fastest under-water elongation. The plants from the third cohort produced slightly less seeds compared with those from the second cohort in the same length class.

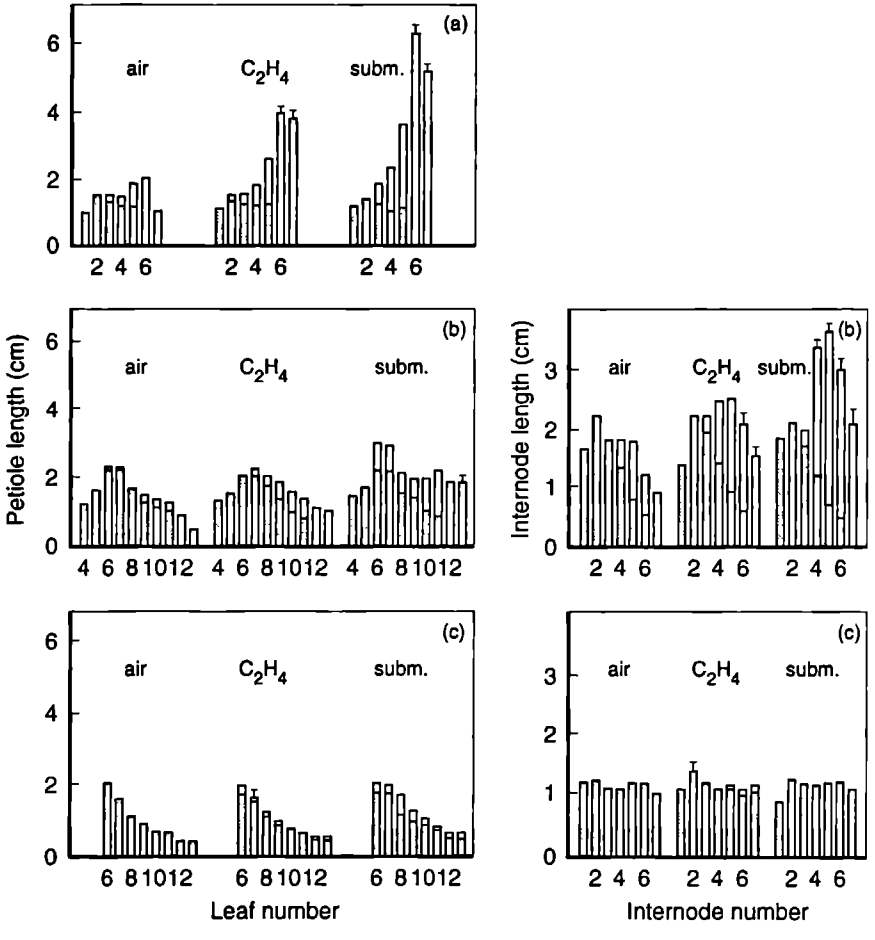


Fig. 2. Mean petiole and internode lengths (±1SE; SE's < 1 mm are not indicated, *n*=8) in *Rumex maritimus*, before (shaded bars) and after (open bars) 4 days of treatment in three stages: (a) rosette; (b) bolting; (c) flowering. Treatments: Air, plants daily flushed with medical air; C_2H_4, plants daily flushed with $5x10^{-6}$ m^3m^{-3} ethylene/air; subm., plants submerged in tapwater. Leaves and internodes are numbered in the order of appearance (the first stretched internode appeared between leaves 6 and 7).

Ethylene experiments

There appeared to be a remarkable difference between three stages of development with respect to the relative elongation response of petioles and internodes upon flooding. In the rosette stage (Fig. 2a), there was a very rapid extension of petioles (laminae are not presented since they showed only a very small [< 1 cm] extension effect). In the bolting stage (Fig. 2b), the extension of petioles (from stem leaves) was much less, and in the flowering stage (Fig. 2c) it was practically non-existent. Internode elongation was large in the bolting stage (Fig. 2b), but absent in the flowering stage (Fig. 2c). These results agree with the results of the outdoor experiment (Fig. 1). The process could be mimicked to a large degree by applying external ethylene (Fig. 2).

A large increase of internal ethylene concentration was measured within 12 h of submergence in the rosette stage (Fig. 3a); thereafter the concentration decreased. In the bolting stage the largest increase was the same, but was reached only after 24 h (Fig. 3b); subsequently it declined as in the former stage. The internal ethylene concentration after 12 h of submergence in the flowering stage was low compared with the earlier stages (Fig. 3c). There was a slight daily fluctuation in the ethylene concentration of drained plants in all three stages, with the higher values being found at the end of the light period.

The results of the measurements of ethylene entrapment and production with the continuously monitoring system are presented in Fig. 4. Upon submergence, the ethylene evolving from a plant of *R. maritimus* decreased at first, indicating entrapment by the water column, but increased after approximately 10 h. After 20 h, more ethylene evolved from the submerged plant through the water column than from the waterlogged plant. At this point (35 h) 50% more ethylene (0.3 nmol $g^{-1}h^{-1}$) was produced by the flooded plant than by the drained plant (0.2 nmol $g^{-1}h^{-1}$). After lowering the water level from submergence to waterlogging conditions two peaks in ethylene production were observed (Fig. 4, inlay). The first and smaller one appeared within 1 h. The top of the second peak, which lasted for about 15 h, was reached after approximately 3 h. Ethylene production remained higher for several days, compared with drained and waterlogged conditions. In spite of the continuous light regime, a diurnal rhythm in the ethylene production level was observed.

Submergence in water containing AVG, an inhibitor of ethylene biosynthesis (data not shown), resulted in a considerably reduced ethylene release from the water, compared with submergence in water without AVG. Practically no rise in ethylene occurred in 24 h and at the end of the submergence period, the ethylene evolving from the plant in AVG was 0.07 nmol $g^{-1}h^{-1}$, thus even less than from drained plants. After lowering the water table, the first ethylene peak was only about three times that of the drained level. This increase was small in comparison with the twentyfold increase after submergence in water. The second peak was completely absent, and ethylene production fell to drained levels 2 h after the small first peak.

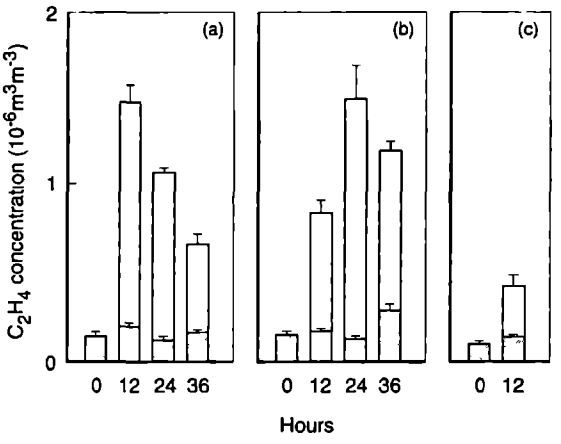


Fig. 3. Mean internal ethylene concentrations (±1SE, 3-4 samples) in *Rumex maritimus* after 0, 12, 24 and 36 h (0 h is 09.00 h) of treatment in three stages: (a) rosette; (b) bolting; (c) flowering. Shaded bars: drained; open bars: submerged in tap-water.

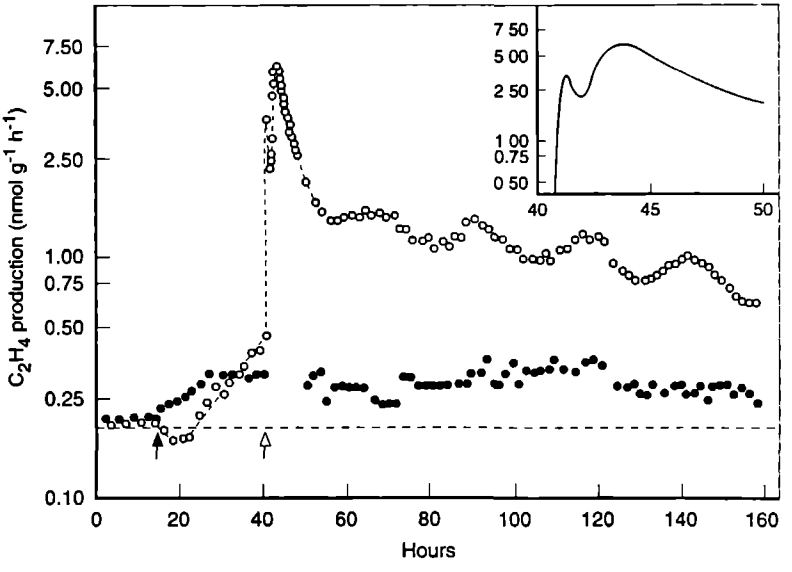


Figure 4. Ethylene production (logarithmically presented) of intact plants of *Rumex maritimus* in the bolting stage, monitored in a continuous flow system. ----- mean ethylene production of drained plants; ● plant waterlogged from ↑onwards; ○ plant submerged from ↑until ↑and thereafter waterlogged. The inlay represents the two peaks in ethylene production occurring immediately after submergence on a different scale. Results are presented in terms of shoot dry weight, determined at the end of the measurements.

Discussion

A shift from extension of petioles to extension of internodes and a decrease of the rapid elongation response upon flooding in the course of the development of a flowering plant was observed in outdoor and laboratory experiments (Figs. 1 and 2). Changing responsiveness of petioles or internodes at different stages of ontogeny is mentioned by Ridge (1987) but most studies have only considered the responses of either petioles or internodes in a restricted phase of growth. Keith, Raskin & Kende (1986) compared deepwater rice with non-deepwater rices and found that the ability of internodes to extend was much the same in all cases but in deepwater rice internode elongation, as well as its ability to respond to flooding, occurred during a much longer period, compared with the other rices. This example stresses the importance of the recognition of different stages in a plant's life in which its response to environmental conditions may vary (see also Chapter 5). The role of ethylene was studied by mimicking the effect of submergence through application of external ethylene, and by measuring internal ethylene concentrations. Externally applied ethylene did not wholly reproduce the extension response of submergence in our experiments (Fig. 2). Métraux & Kende (1984) found that only ethylene in combination with a gas mixture containing low oxygen and high carbon dioxide concentrations could entirely reproduce the submergence effect in the internodes of deepwater rice. An enhancement of the ethylene effect by a high CO_2 concentration, probably occurring in submerged tissue, was also found by Raskin & Kende (1984). Another factor that cannot be reproduced by exogenous ethylene is the buoyant tension of the water column, which may play an additional role in the submergence response (Jackson 1985; Ridge 1987).

Internal ethylene concentrations, measured in excised plant parts, increased within 12 h of submergence (Fig. 3). Results in the rosette stage were quite similar to those obtained for other *Rumex* species (Voesenek & Blom 1989a). Ethylene concentrations in the stem elongation stage are also in agreement with values obtained for other genera (*e.g.* Métraux & Kende 1983). After 12 h of submergence in the flowering stage, the ethylene concentrations were lower than those of the previous stages. Differences in kinetics and/or amounts of accumulated ethylene could be important for the changing response of the elongating tissues during ontogeny and should therefore be studied in more detail.

With the photoacoustic technique we measured the ethylene evolving from an intact plant before, during and after a period of submergence. Upon submergence ethylene was entrapped, since gas diffusion was effectively impeded by the surrounding water. However, even within a relatively short time (24 h), increasing amounts of ethylene evolved from the water surface, indicating a high internal ethylene concentration (Fig. 4). After lowering the water to soil level, the accumulated ethylene diffused rapidly from the plant, resulting in a peak of ethylene within an hour. The amount of entrapped ethylene during the 24-h period of submergence, the amount of released ethylene during the last hours of submergence, and the first peak were compared with the ethylene production under waterlogged conditions. We computed that twice as much ethylene was released as could have been entrapped during submergence if the production level was the same as under waterlogged conditions. This suggests that the ethylene production was higher during submergence than during waterlogging. The application of AVG during the submergence treatment dramatically reduced the ethylene output, which may be a further

indication of enhanced ethylene production during the 24-h submergence period. After lowering the water level, a small amount of accumulated ethylene evolved from the plant treated with AVG. Thus, increased production as well as entrapment of ethylene appear to be important during submergence in *R. maritimus*.

A second, much larger ethylene peak appeared a few hours after the first following submergence in water (Fig. 4). This second peak was completely absent following submergence in AVG. The time course of ethylene release we observed suggests that accumulated ethylene was released (first peak), while accumulated ACC was converted to ethylene immediately after lowering the water level (second peak). Woltering & Harren (1989) applied ACC to orchid flowers and found a comparable large ethylene peak with the top appearing after approximately 4 h. Increase in ethylene biosynthesis in submerged tissue is due to enhanced ACC-synthesis rather than increased conversion of ACC to ethylene (Métraux & Kende 1983; Cohen & Kende 1987). Thus probably only part of the extra synthesized ACC was converted to ethylene during the submergence period. The ecological advantage of this could be that even after restoration of shoot-atmosphere contact and the dissipation of entrapped ethylene into the atmosphere, rapid growth is continued by enhanced ethylene synthesis from accumulated ACC, allowing a larger portion of the plant to emerge from the water.

In the outdoor experiment, survival of plants of *R. maritimus* during and after a flooding period of 4 weeks was largely dependent on their ability to emerge with part of the shoot above the water surface. Vegetative shoots were unable to reach the surface and died within 2 weeks. Evidence that elongation of petioles is of importance for the survival of vegetative plants of *R. crispus* and *R. palustris* is presented by Voesenek (1990). A functional significance of the petiole elongation response was found in *R. maritimus* under laboratory conditions by Laan *et al.* (1990). The amount of internal aeration was positively correlated with the leaf area protruding from the water. In the present study we also found a positive relationship between seed output and stem elongation in flooded plants of *R. maritimus*. Flowering plants which remained under water for 4 weeks were dead afterwards and produced only a small amount of seeds (probably originating from flowers that were pollinated before the onset of flooding). Plants submerged prior to flowering were able to elongate under water. In general, it appeared that the plants least developed, yet beyond the rosette stage, could extend a larger part of the shoot above the water surface and produce more seeds (Table 3).

The performance of *R. maritimus* in river forelands is dependent on flooding regimes (see *e.g.* Chapter 2; Van der Sman, Joosten & Blom 1993, Chapter 4 in this thesis), which are largely unpredictible (Blom *et al.* 1990; Voesenek 1990). Roberts & Boddrell (1985) studied the viability of seeds and time of emergence of seedlings for many grassland and ruderal species. They found for *R. maritimus* relatively short-lived seeds with main emergence in the first spring after sowing. Very little emergence occurred after the end of May. In several outdoor experiments in which we applied different flooding regimes (Chapters 3-5), we found that plants of *R. maritimus* flowered under drained conditions when germinated before the end of June, but those which germinated in April and May produced most seeds. Flooding in the rosette stage either resulted in the death of plants or, in case they survived, in delayed flowering and sometimes even the postponement of flowering until the following growing season. In this study, a 10-day delay in flowering resulted in a reduced seed output for the third cohort of flooded plants, compared with plants from the second cohort in the same length category, which flowered in a longer photoperiod (Table 3). Since flooding will also delay germination in *R. maritimus* until after June in several years, there are many occasions in which this species is

forced to remain vegetative in the year of seedling emergence as a result of flooding (Chapters 3-6). Rosettes of *R. maritimus* appeared to be much better capable to survive submergence during the winter than in the summer. Plants flooded during the winter months (the usual situation in their natural habitat) exhibited rapid stem elongation in early spring until a large part of the shoot emerged from the water surface (Chapter 6). Eventually, these plants produced more seeds in the second year of growth than non-winter flooded controls, a further indication of the important role of rapid stem elongation with respect to seed production in this species.

In conclusion, we suggest that both ethylene entrapment during submergence and the enhancement of ethylene production during and especially after submergence, are responsible for the rapid elongation of shoots in *R. maritimus*, which continues after the water surface is reached. Important fitness components, such as survival and seed production, may depend on this process, which appears to determine the flooding resistance of this species (see also Chapters 5 and 6).

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CHAPTER 8

GENERAL DISCUSSION

Summary of the main results

Chenopodium rubrum L., *Rumex maritimus* L. and *Rumex palustris* Sm. differed in their resistance towards flooding which was related to different life histories. The effect of flooding on growth, survival and reproduction of plants occurring in river forelands may vary according to the highly variable flooding regimes (Chapters 1 and 2). Therefore, the different strategies of the three species are summarized here in relation to the main effects of flooding.

Time of establishment

Delay of germination due to late subsidence of winter high-waters or due to flooding in the growing season caused plants of the short-day species *C. rubrum* to flower at an earlier stage of development (Chapters 3 and 4). In later cohorts of this species, less seeds were produced per plant than in early cohorts. However, a higher proportion of resources was allocated to seeds and, in addition, larger seeds which were less dormant were produced in later cohorts.

In order to flower in the year of germination, both long-day species of *Rumex* needed a minimum leaf number before a certain photoperiod had passed (Chapters 3 and 4). Delay of germination resulted in a progressive reduction of the number of flowering shoots per plant and, as a consequence, in a reduced seed output in these species. Gradually, more energy was invested in vegetative growth until after a certain photoperiod flowering was definitely postponed to the following growing season. In *R. palustris*, postponement of flowering occurred in earlier cohorts than in *R. maritimus* due to a larger photoperiodic requirement. In *R. maritimus*, there was a trade-off between seed number and seed size as observed in *C. rubrum*, but this was not associated with differences in dormancy level.

Growth in relation to flooding

Upon flooding in the growing season, established plants of *C. rubrum* exhibited some characteristic features of aquatic plants which may be considered as "adaptive" under conditions of partial submergence, such as the transition from prostrate to vertical growth, adventitious rooting and the formation of aerenchyma in roots and stems (Chapter 2). Chlorosis and death occurred in old leaves first in *C. rubrum* under conditions of partial submergence only, whereas in both *Rumex* spp. this also occurred upon complete submergence (Chapter 4). During total submergence, old as well as young leaves died at random in *C. rubrum* and probably as a consequence, plants had difficulties with regeneration of shoots afterwards. Under conditions of repeated partial submergence, much less adventious roots were initiated and more died in the periods between subsequent floods compared with one continuous flooding period of the same total duration, resulting in reduced biomass and seed output in both *C. rubrum* and *R. maritimus* (Chapter 2). However, *C. rubrum* was much more sensitive in comparison to both *Rumex* spp. under the severest conditions, such as early, intermittent, and deep flooding (Chapters 2, 4 and

5). Water temperatures appeared to play an important role in the tolerance of total submergence in *C. rubrum* as well as in both *Rumex* spp. (Chapters 4-6).

In addition to leaf reorientation, both *Rumex* spp. possessed another important feature of aquatic plants which is important for survival of submergence. Upon flooding, they were capable of rapid elongation of petioles and stems in order to regain contact with the atmosphere (Chapters 5-7). Adventitious roots were initiated after restoration of shoot-air contact. More adventitious roots of higher porosity were inititated under conditions of partial submergence in *R. maritimus* compared with *C. rubrum*. *R. maritimus* appeared to be highly dependent on these mechanisms aimed at avoidance of anaerobiosis in order to survive under conditions of submergence (Chapters 5-7). *R. palustris* could survive longer periods of total submergence and was the most flooding-tolerant species with respect to survival and plant size after different conditions of flooding (Chapters 4-6).

Reproduction in relation to flooding

Delay of flowering was found after flooding under long days in the greenhouse in *C. rubrum* (Chapter 2). This was probably caused by the larger size-requirement in long days than in short days in this species (Chapters 3 and 4). Under field conditions, delay of flowering due to flooding is therefore probably unimportant in *C. rubrum*. Seed output, however, may be severely reduced in *C. rubrum* as a result of flooding due to: (i) reduction of plant biomass, (ii) destruction of flowers, and (iii) the inability of regeneration of flowering shoots (Chapters 2 and 4). In both *Rumex* spp., flooding in the growing season frequently resulted in delay of flowering (Chapters 4 and 5). Leaves were initiated at a lower rate during flooding and the minimum leaf number was therefore reached later in the season under shortening photoperiods. Especially in *R. palustris*, postponement of flowering until the following growing season was frequently observed.

Seed output depended on growth characteristics during flooding in both *Rumex* spp. and most seeds were produced by plants which emerged with a large part of the flowering shoot above the water surface (Chapters 5-7). However, large stems also appeared to have a metabolic cost. Differences between both species in seed output in the first growing season were probably caused by larger stems in *R. palustris* than in *R. maritimus* under both drained and flooded conditions. This feature may be important for the survival of deeper inundations in the growing season. Delay of flowering in the first growing season may also contribute to flooding resistance in *R. palustris*. Plants of this species which suffered from submergence in early summer, but were able to recover for some time afterwards before flowering, also produced relatively large amounts of seeds (Chapter 5). Submergence of flowering shoots not only resulted in severe reduction of flowers as in *C. rubrum*, but also in seeds of low quality in *R. maritimus* (Chapter 4). Plants of both *Rumex* spp., however, were better able than *C. rubrum* to regenerate new shoots after submergence and this ability was not lost after flowering (Chapters 4-6). As a consequence, the seed output of plants of *Rumex* was large after flooding, compared with *C. rubrum* (Chapters 2 and 4).

Longevity in relation to flooding

In the strictly annual species *C. rubrum*, winter submergence may act to determine the end of the growing season (Chapter 1). In both *Rumex* species, survival of winter high-waters was necessary in cohorts which remained vegetative in the first growing season due to late germination and/or flooding in the rosette stage, in order to reproduce in the following season.

In river forelands, the biennial life history appeared to occur even more frequently in these species than the annual strategy (Chapter 6). In *R. palustris*, short-lived perennality was also observed in river forelands and under experimental conditions, by means of vegetative proliferation after flowering. Plants of both *Rumex* spp. of different ages and sizes were able to survive 4 months of submergence during winter and early spring (Chapter 6). Petiole and internode elongation in early spring resulting in shoot emergence, enhanced survival and initiated reproductive development. Seed output was relatively large in emergent plants compared with plants which remained submerged during the winter or were kept drained.

Under a variety of flooding regimes in outdoor experimental plots, the most flooding-tolerant species *R. palustris* produced relatively more seeds in following growing seasons than in the first season, compared with *R. maritimus*, while the most sensitive species *C. rubrum* produced all of its seeds in the first season.

The role of ethylene in shoot elongation

The role of the gaseous hormone ethylene was established in the under-water shoot elongation responses in *R. maritimus* (Chapter 7). High internal or externally applied ethylene levels resulted in extension of petioles in the rosette stage and of petioles and/or internodes in the bolting stage. Upon submergence, entrapment of ethylene by the water layer followed by increased production levels were measured in a flow-through system with a photoacoustic detection apparatus. Immediately after lowering the water table to waterlogging level, entrapped ethylene was released followed by a second increase in ethylene production which was probably caused by aerobic conversion of the entrapped precursor ACC. The ecological significance of this increased production of ethylene after emergence may be that fast growth is continued until larger shoot parts protrude from the water surface, which increased the fitness of plants of *R. maritimus* (Chapters 5-7).

Flooding resistance and life histories

Many studies have considered flooding resistance in terms of the ability of seeds, seedlings, and established plants to deal with oxygen stress (*e.g.* Hook & Crawford 1978; Kozlowski 1984; Crawford 1987; Voesenek 1990; Laan 1990; Jackson, Davies & Lambers 1991). Levitt (1972) distinguished two major pathways in plants to resist stresses of different kinds: avoidance and tolerance. With respect to resistance of anoxia, these pathways were called "apparent" and "true" tolerance to anoxia by Vartapetian (1978). Although Levitt (1972) as well as many other authors discussed changes in resistance towards several kinds of stress in the course of plant development and often found seeds to be much more tolerant towards them than established plants, he treated both avoidance and tolerance as purely physiological mechanisms on the individual plant level. In terms of life-history strategies, high tolerance in the seed phase or other dormant stages (*e.g.* Hook 1984; Braendle & Crawford 1987) may be interpreted as a mechanism of avoidance of the stress during more sensitive parts of the established phase (see Harper 1977; Grime 1979; Tilman 1988). Fitter & Hay (1981) used the term tactic to distinguish between different mechanisms of stress resistance. Different tactics *sensu* Fitter & Hay may be included in a life-history tactic as "a set of co-adapted traits" according to Stearns

(1976). Fitter & Hay (1981) distinguished three primary tactics which may occur in combination: avoidance, amelioration and tolerance. The term avoidance as used by Fitter & Hay designates the tactic of dormancy, which is interpreted as stress tolerance by Levitt (1972). Animals may simply walk away, but plants may typically use dormant stages to deal with predictable stresses, such as winter flooding in river forelands (Voesenek & Blom 1992a). The tactic of amelioration *sensu* Fitter & Hay (1981) is the same as the mechanism of avoidance according to Levitt (1972): plants confront the environmental factor which causes stress but alleviate its adverse effects. Perfect examples of this tactic are the shoot elongation responses exhibited by *Rumex maritimus* and *R. palustris* upon flooding, followed by adventitious rooting which serve to alleviate oxygen stress. Tolerance towards stress is interpreted as real metabolic tolerance by both Levitt (1972) and Fitter & Hay (1981) but Levitt also classifies reparability under this notion. Distinction between tolerance and avoidance mechanisms are probably most useful if the level of organization is clearly defined beforehand. Different classifications may arise from different viewpoints. If we consider that an environmental stress may be experienced as a factor of disturbance (*sensu* Grime 1979), depending on the longevity of plants, the importance of considering life histories becomes evident. Menges & Waller (1983) discussed this in relation to the occurrence of both stress tolerators and ruderals in the most frequently flooded areas of floodplains, but also in extremely dry places these two strategies may occur (see *e.g.* Harper 1977; Grime 1979).

Different life-history theories agree on the important role of the seedbank as a genetically determined life-history strategy in areas of high disturbance (*e.g.* MacArthur & Wilson 1967; Grime 1979; Tilman 1988). *C. rubrum* possessed most ruderal characteristics as listed by Grime (1979), including predation by unspecialized animals, such as rabbits (Blom *et al.* 1990). Phenotypic plasticity in response to small-scale environmental fluctuations (Bradshaw 1965; Fitter & Hay 1981), was mainly expressed in reproductive characteristics in *C. rubrum*, such as the onset of flowering, seed size and seed dormancy, which is characteristic for very short-lived species. Seed-polymorphism appears to be a bet-hedging tactic in *C. rubrum* and related species (Harper, Lovell & Moore 1970). *R. palustris* possessed most characteristics of stress tolerators as listed by Grime (1979), such as a longer life history and vegetative expansion, predation by specialized beetles, a relatively large storage organ (tap root), and large seeds in comparison to *R. maritimus* and *C. rubrum*. Environmental fluctuations resulted in less variation in reproductive characteristics and higher plasticity in growth responses towards flooding in *Rumex* compared with *C. rubrum*. In terms of MacArthur & Wilson (1967), *C. rubrum* is the most productive and *R. palustris* the most efficient of the three species under study. *C. rubrum* behaves as a relatively unspecialized, more or less permanently colonizing species, while *R. maritimus* and *R. palustris* appear to be increasingly specialized to the prevailing conditions of flooding in their habitats.

In conclusion, avoidance and tolerance mechanisms were distinguished in the studied species on different levels of organization. Avoidance of severe flooding on the life-history level occurred in all three species by means of tolerant seeds in the soil.

In addition, *C. rubrum* may resist less severe flooding regimes during the established phase through morphological adaptations. Real flooding tolerance is probably restricted to the seed phase, and in terms of life-history strategy this strictly annual species should be considered as mainly avoidant according to Fitter & Hay (1981).

Flooding tolerance is also restricted to the seed phase in *R. maritimus*. In the established phase, *R. maritimus* aimes at alleviating oxygen-stress through developmental adaptations which is

called avoidance by Levitt (1972) and amelioration by Fitter & Hay (1981). Dormancy of vegetative plants of *R. maritimus* during the harsh winter season occurs as an additional mechanism of avoidance according to Fitter & Hay. On the life-history level, the mostly biennial species *R. maritimus* is more flooding tolerant than *C. rubrum* since established plants can resist several more severe conditions of flooding.

R. palustris possesses the same mechanisms of flooding resistance as *R. maritimus*, but in addition real flooding tolerance occurs in the established phase (*sensu* Levitt 1972; Fitter & Hay 1981). Avoidance of the strain caused by oxygen deficiency probably occurs on the cel level (metabolic). The ability of regrowth after flooding is high in both species of *Rumex* compared with *C. rubrum* and highest in the short-lived perennial species *R. palustris*. Consequently, on both the individual plant level as well as on the life-history level, *R. palustris* is the most flooding-tolerant species.

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OVERSTROMINGSRESISTENTIE EN DE LEVENSCYCLUS VAN KORTLEVENDE PLANTEN IN OVERSTROMINGSVLAKTEN

In dit proefschrift zijn de resultaten neergeschreven van onderzoek naar de overstromingsresistentie van enkele kortlevende kruidachtige soorten in relatie tot belangrijke kenmerken van de levenscyclus. De rode ganzevoet (*Chenopodium rubrum* L.), de goudzuring (*Rumex maritimus* L., voorheen zeezuring genaamd) en de moeraszuring (*Rumex palustris* Sm.) zijn vertegenwoordigers van laaggelegen, vaak overstroomde standplaatsen in de uiterwaarden van de grote Nederlandse rivieren. De zuringsoorten komen met name op moddervlakten en in drooggevallen oude rivierlopen tot expressie, terwijl de rode ganzevoet typerend is voor vloedmerken op zandstranden langs de rivier. De drie soorten zijn ook te vinden in de veelvuldig voorkomende afgravingen, zoals kleiputten. Al deze laaggelegen terreinen worden gekenmerkt door min of meer voorspelbare, langdurige en diepe overstromingen in de winter en relatief korte overstromingen in het groeiseizoen, welke laatste onvoorspelbaar zijn wat betreft tijdstip en frequentie van voorkomen, duur en diepte (zie tabel 1 in hoofdstuk 1). Met name deze onregelmatig optredende overstromingen in het groeiseizoen oefenen hun invloed uit op belangrijke levensprocessen zoals vestiging van kiemplanten, vegetatieve groei, bloei en zaadproductie. De drie genoemde soorten kunnen de voorspelbare winteroverstromingen overleven door middel van een zaadbank, bestaande uit grote hoeveelheden langlevende en overstromingsresistente zaden die bijzondere kiemeigenschappen hebben, zodat kieming pas optreedt na het droogvallen van de bodem. Een dergelijke strategie is kenmerkend voor soorten van natte standplaatsen.

Aangezien overstromingsresistentie alleen kan worden beschouwd in relatie met de in het uiterwaardengebied zeer wisselende waterstanden (hoofdstukken 1 en 2), zijn de overlevingsstrategieën van de rode ganzevoet, de goudzuring en de moeraszuring bestudeerd onder verschillende, kenmerkende overstromingsregimes. Een belangrijk deel van het onderzoek werd uitgevoerd in proefvelden waarin de waterstand gereguleerd kon worden (zie fig. 1 in hoofdstuk 1). Naast onderzoek onder min of meer natuurlijke omstandigheden, zijn aanvullende oecofysiologische studies verricht in kas en laboratorium.

Tijdstip van vestiging

In hoofdstuk 3 is de invloed van het tijdstip van kieming en vestiging op de reproductieve ontwikkeling van de drie soorten aan de orde gekomen. Winterhoogwaters kunnen zich reeds vroeg in het voorjaar terugtrekken, maar kunnen zich ook handhaven tot ver in de zomer. Bovendien kunnen nieuwe kiemingsgolven optreden na afloop van voorjaars- en zomeroverstromingen. Meerdere cohorten (groepen min of meer gelijktijdig gekiemde planten) kunnen aldus naast en na elkaar voorkomen. De rode ganzevoet is een strikt eenjarige soort die de levenscyclus in één groeiseizoen voltooit. Bloei-inductie geschiedt door korte fotoperioden (korte-dag bloei-inductie) en uit dit onderzoek bleek dat er bij deze soort laat in het seizoen nauwelijks een minimale grootte noodzakelijk was voor bloei optrad. In lange dagen was dit wel het geval. Als gevolg hiervan kwamen latere cohorten van de rode ganzevoet relatief snel tot bloei en bovendien als kleinere planten vergeleken met vroege cohorten. Planten in latere

cohorten investeerden relatief meer biomassa (gewicht) in zaden dan in stengels en wortels en produceerden minder, grotere zaden. Deze zaden waren minder dormant dan de kleinere zaden van eerder gekiemde planten. De productie van zaden van verschillende grootte en met verschillende kiemeigenschappen is vermoedelijk een onderdeel van de overlevingsstrategie van deze zeer kortlevende soort.

De beide rozetvormende zuringsoorten kwamen alleen onder lange-dag condities in bloei en hadden bovendien een bepaalde grootte nodig om tot bloei te kunnen komen. Dit laatste uitte zich vooral als een minimum aantal bladeren per (hoofd- of zij-)scheut. Bij vroeggekiemde planten kwamen de hoofdscheut en alle okselscheuten van rozetbladeren in bloei, bij later gekiemde exemplaren kwamen steeds minder zijscheuten in bloei en bij nog latere cohorten bleef de plant helemaal vegetatief in het eerste groeiseizoen. De niet-bloeiende zijscheuten van rozetbladeren waren in staat te overleven nadat de hoofdscheut was afgestorven na zaadzetting.

De moeraszuring had langere dagen nodig om tot bloei te geraken dan de goudzuring en vermoedelijk ook een meer specifieke temperatuurbehoefte. Verlate kieming had daarom eerder het uitstel van bloei tot het volgende groeiseizoen tot gevolg bij de moeraszuring.

Ook bij de zuringsoorten bestond er een trade-off (omgekeerd evenredig verband) tussen aantallen zaden en zaadgrootte per plant maar dit ging niet gepaard met verschillen in dormantie als bij de rode ganzevoet (hoofdstukken 3 en 4).

Groei in relatie tot overstroming

Gevestigde planten van de rode ganzevoet vertoonden bij inundatie (overstroming) enkele voor moerasplanten karakteristieke eigenschappen (hoofdstukken 2 en 4). Bladeren en zijtakken veranderden van een horizontale naar een verticale oriëntatie en wanneer hierdoor een deel van de scheut boven water uitstak, werden adventiefwortels gevormd op het scheidingsvlak van stengel en penwortel en op het deel van de stengel dat zich onder water bevond. In beperkte mate werd luchtholteweefsel (aerenchym) gevormd in stengels en wortels. De rode ganzevoet was op deze wijze enigszins aangepast aan alleen wortelinundatie of inundatie van slechts een deel van de scheut. In geval van algehele onderdompeling hadden planten van de ganzevoet geen profijt van deze aanpassingen en ging bovendien een andere aanpassing verloren. Dit betreft het verschijnsel dat oude bladeren tijdens overstroming afsterven ten gunste van jonge bladeren en scheuten. Bij de rode ganzevoet gebeurde dit alleen bij gedeeltelijke overstroming terwijl het bij beide zuringsoorten ook bij volledige onderdompeling voorkwam. De rode ganzevoet vertoonde dan ook een veel grotere sterfte bij volledige onderdompeling en was ook overigens gevoeliger onder verschillende overstromingsregimes (hoofdstukken 2, 4 en 5). Een belangrijke rol bij de tolerantie van totale onderdompeling speelde de watertemperatuur bij alle drie de soorten (hoofdstuk 4).

De zuringsoorten bezaten een additionele eigenschap van water- en moerasplanten die van belang was voor overleving van diepere inundaties. Bij onderdompeling hadden zij door middel van snelle strekkingsgroei van petiolen (bladstelen) en internodiën (stengelleden) de mogelijkheid om boven het wateroppervlak uit te groeien (hoofdstukken 5-7). Net als de rode ganzevoet vormden de zuringen pas adventiefwortels nadat het contact tussen de scheut en de atmosfeer hersteld was. Bij de zuringen werden meer en dikkere adventiefwortels gevormd van een hoge porositeit, zowel vanuit de penwortel, de overgang penwortel en spruit, als vanuit de stengelknopen. De goudzuring bleek in hoge mate afhankelijk van deze mechanismen die leiden tot (gedeeltelijk) herstel van het zuurstoftekort veroorzaakt door overstroming (hoofdstuk 5). De moeraszuring was onder verschillende omstandigheden steeds het best en het langst in staat tot

het overleven van totale onderdompeling en bleek dus het meest tolerant ten aanzien van zuurstofgebrek in vergelijking met beide andere soorten (hoofdstukken 4-6).

Bloei en zaadproductie in relatie tot overstroming

Bij de korte-dag plant, de rode ganzevoet, leidde overstroming in het groeiseizoen onder natuurlijke omstandigheden nauwelijks tot uitstel van bloei (hoofdstuk 4). De zaadproductie werd er wel ernstig door gereduceerd, indirect als gevolg van een geringere biomassa per plant en direct door vernietiging van bloemen (hoofdstukken 2 en 4). Omdat deze soort bovendien geen bloeiende scheuten kon regenereren na overstroming was de zaadproductie na overstroming veelal zeer laag.

Als gevolg van de noodzaak van een minimum plantgrootte in combinatie met lange-dag inductie, resulteerde overstroming bij de beide zuringsoorten en met name bij de moeraszuring nogal eens tot uitstel van bloei tot het volgende groeiseizoen (hoofdstukken 4 en 5). Bij deze soorten was de zaadproductie in het eerste groeiseizoen afhankelijk van de groeirespons tijdens overstroming (hoofdstuk 5). Het meeste zaad werd gevormd door planten die ver boven water uit staken met de bloeistengels. Het maken en onderhouden van lange stengels kost echter ook biomassa en energie en dit uitte zich niet alleen tussen de twee soorten maar ook binnen de soorten: de moeraszuring produceerde altijd langere stengels en navenant minder zaad dan de goudzuring bij een vergelijkbare biomassa en planten van beide soorten die extreem ver boven water uit groeiden produceerden minder zaad dan iets kortere planten. Deze trade-off tussen stengels en zaad, die bij de moeraszuring meer in het voordeel van de stengels lag, kan de overlevingskansen van deze soort verhogen in geval van diepe inundaties in het groeiseizoen.

Uitstel van bloei bleek eveneens van belang te kunnen zijn voor de zaadproductie van de moeraszuring. Indien planten van deze soort na afloop van een voorjaarsoverstroming een herstelperiode doormaakten voordat ze in bloei kwamen, was hun zaadproductie hoger dan van planten die voor inundatie weliswaar groter waren maar die als gevolg daarvan ook eerder bloeiden, zonder herstelperiode na afloop van de overstromingsperiode (hoofdstuk 5).

De zuringsoorten bezaten in vergelijking met de rode ganzevoet de gunstige eigenschap van doorlopende regeneratie van bloeiende scheuten in de oksels van rozet- en stengelbladeren. Daardoor kon ook na overstroming van bloeiende planten van de goudzuring nog een relatief hoge zaadproductie optreden (hoofdstuk 4). Een relatief hoge zaadproductie ging onder deze condities echter wel gepaard met een lage zaadkwaliteit.

Levensduur in relatie tot overstroming

Winteroverstroming speelde bij de rode ganzevoet alleen een rol door het bepalen van het einde van het groeiseizoen (hoofdstuk 1). Voor beide zuringsoorten was het overleven van winterhoogwaters van belang voor de latere cohorten en voor de in het groeiseizoen geïnundeerde eerdere cohorten, die vegetatief bleven in het eerste groeiseizoen. Bij inventarisatie van verschillende levensduren in het rivierengebied bleek zelfs dat de tweejarige strategie veel vaker voorkwam dan de eenjarige bij deze zuringsoorten (hoofdstuk 6). Bij de moeraszuring was eenjarigheid in de praktijk zeldzaam en trad bovendien perenniteit (meerjarigheid) op door middel van vegetatieve vermeerdering na de bloei.

Beide zuringsoorten bleken in staat tot overleving van maandenlange totale onderdompeling in de winter en het vroege voorjaar, zelfs als heel kleine planten (hoofdstuk 6). Dormantie speelde hierbij vermoedelijk de hoofdrol, maar relatief lage watertemperaturen in de winter droegen er ook toe bij. Het proces van bladsteel- en stengelstrekking onder water werd opnieuw van belang

voor de overjarige planten in het vroege voorjaar. Het maken van atmosferisch contact was bevorderlijk voor de overleving van het winterhoogwater en zette de reproductieve ontwikkeling in gang. Deze verliep veel sneller in volgende seizoenen dan in het eerste en de zaadproductie van planten die boven water uitkwamen in het vroege voorjaar was niet alleen hoger dan die van planten die onder water bleven, maar ook hoog in vergelijking met planten uit dezelfde cohorten die geen winteroverstroming hadden meegemaakt.

Onder een aantal verschillende regimes van overstroming produceerde de meest overstromingstolerante soort, de moeraszuring, steeds in verhouding de meeste zaden in volgende groeiseizoenen vergeleken met de goudzuring. Aan het andere uiterste bevond zich de overstromingsgevoelige rode ganzevoet die uitsluitend in het eerste groeiseizoen zaad produceerde.

De rol van ethyleen bij strekkingsprocessen onder water

De rol van het gasvormige plantehormoon ethyleen (etheen) bij de snelle strekkingsgroei van bladstelen en stengelleden onder water is beschreven in hoofdstuk 7. Externe toediening van ethyleen in hoge concentraties kon het strekkingseffect gedeeltelijk nabootsen en bij ondergedompelde planten werd een verhoogde interne ethyleenconcentratie aangetoond.

De ethyleen-evolutie van een intacte plant werd gemeten in een gesloten doorstroomsysteem van medicinale lucht met behulp van een in de afgelopen jaren ontwikkelde lasergestuurde fotoakoestische meettechniek. In geval van een ondergedompelde plant werd ethyleen gemeten in de luchtstroom die langs het wateroppervlak voerde. De resultaten van dit onderzoek wezen er op dat als gevolg van totale onderdompeling de concentratie van ethyleen in de plant toenam, enerzijds door ophoping omdat de diffusie van dit gas in water veel geringer is dan in lucht, anderzijds door een toename van de interne productie van het hormoon. Na verlaging van de waterspiegel tot het niveau van wortelinundatie kwam het opgehoopte ethyleen onmiddellijk vrij, waarna een nieuwe stijging van de ethyleenproductie werd gemeten. Deze werd toegeschreven aan de gevolgen van het vrijkomen van in de plant opgehoopt ACC (voorloper van ethyleen in de ethyleenstofwisseling). Voor de omzetting van ACC in ethyleen, de laatste stap in de ethyleenbiosynthese, is zuurstof nodig en het is zeer goed denkbaar dat deze stap geremd was gedurende de periode van totale onderdompeling.

Het oecologisch belang van deze verhoogde ethyleenproductie na herstel van atmosferisch contact bij totale inundatie of bij konstante wortelinundatie zou kunnen zijn dat het snelle strekkingsproces voortduurt tot een zo groot mogelijk deel van de scheut boven water uitsteekt. Interne aeratie van het (geïnundeerde) wortelstelsel is namelijk afhankelijk van de relatieve scheutoppervlakte boven water en ook de uiteindelijke zaadproductie was positief gecorreleerd met de scheutlengte boven water tijdens inundatie (hoofdstukken 5 en 6).

Overstromingsresistentie in relatie tot de levenscyclus

In hoofdstuk 8 zijn de resultaten van de eerdere hoofdstukken samengevat en de verschillende eigenschappen met betrekking tot overstromingsresistentie van de drie soorten gerelateerd aan (de plasticiteit van) hun levensloop. De conclusie is dat overstromingsresistentie wordt bepaald door de som van mechanismen van het ontwijken van overstroming of de gevolgen ervan (avoidance) en het tolereren ervan (tolerance), welke op verschillende niveau's van organisatie kunnen optreden. Op het niveau van de levenscyclus zijn deze soorten, zoals eerder genoemd, ontwijkers: door middel van een zaadreserve van dormante en overstromingsresistente zaden (zaadbank) ontlopen zij met name de voorspelbare, ongunstige wintersituatie. Deze genetisch

vastgelegde strategie is algemeen voor soorten van standplaatsen waar veel verstoring optreedt en dus regelmatig hervestiging nodig is. Daarnaast hebben de drie soorten echter in toenemende mate eigenschappen om ook in de gevestigde fase van de levenscyclus overstroming te "tolereren". Deze eigenschappen treden voornamelijk op in de vorm van fenotypische plasticiteit (variatie in verworven eigenschappen, die overigens ook onder genetische controle staat).

De rode ganzevoet kan tijdens de gevestigde fase alleen door middel van een beperkt aantal morfologische aanpassingen aan de minder ernstige vormen van overstroming weerstand bieden. Een grote mate van plasticiteit treedt op met betrekking tot reproductieve parameters, zoals de duur van de levenscyclus, die echter steeds binnen één groeiseizoen wordt voltrokken, en zaadaantal en -grootte. Deze soort is dus voornamelijk als een ten opzichte van inundatie weinig gespecialiseerde ontloper van ongunstige condities te beschouwen.

Ook de goudzuring is alleen in de zaadfase echt overstromingstolerant. In de gevestigde fase bezit deze soort mechanismen van fenotypische plasticiteit die gericht zijn op het herstel van zuurstofgebrek, zoals strekkingsprocessen in de scheut, adventiefworteling en luchtholtevorming. Bovendien kunnen gevestigde planten in dormante conditie een langdurig winterhoogwater overleven. Op het niveau van de levenscyclus is de meestal tweejarige goudzuring dus meer tolerant dan de rode ganzevoet en meer gespecialiseerd ten aanzien van overstroming.

De moeraszuring bezit grotendeels dezelfde aanpassingen als de goudzuring, maar kan als gevestigde plant bovendien het best en langdurigst totale onderdompeling tolereren, hetgeen veelal gepaard gaat met uitstel van bloei. Dit wijst op aanpassing van stofwisselingsprocessen die er op celniveau weer op gericht zijn schade te vermijden. De mogelijkheden tot hergroei van zowel vegetatieve als generatieve plantedelen zijn groot bij de beide zuringsoorten vergeleken met de rode ganzevoet en het grootst bij de moeraszuring. Dus zowel op het niveau van de individuele plant als op het niveau van de levenscyclus is de kortlevend perenne soort, de moeraszuring, het meest overstromingstolerant.

CURRICULUM VITAE

Jeannette van der Sman werd geboren op 14 maart 1957 te Nootdorp. Zij doorliep het VWO aan het St. Maartenscollege te Voorburg en behaalde in 1975 het diploma Atheneum-b. In de daarop volgende jaren deed zij verschillende studie- en werkervaringen op. Haar belangstelling voor de biologie werd gewekt tijdens de opleiding tot biologisch analist op het Van Leeuwenhoek Instituut te Delft.

In 1979 begon zij met de studie biologie aan de faculteit Wiskunde en Natuurwetenschappen van de Katholieke Universiteit van Nijmegen, in de doctoraalfase gecombineerd met filosofie op kandidaatsniveau. In 1986 behaalde zij *cum laude* het doctoraalexamen biologie, met als hoofdrichting Experimentele Plantenoecologie bij prof.dr. C.W.P.M. Blom en als bijrichtingen palynologie bij prof.dr. D. Teunissen en fysische geografie bij prof.dr. J.J.C. Piket.

Van 1 september 1986 tot 1 december 1990 werkte zij in het kader van een deeltijdaanstelling aan het in dit proefschrift beschreven promotie-onderzoek. Het onderzoek werd uitgevoerd binnen de huidige werkgroep Experimentele Plantenoecologie (Vakgroep Oecologie) onder leiding van prof.dr. C.W.P.M. Blom en gesubsidieerd door BION(NWO). Voor de eerste publicatie die in het kader van dit onderzoek verscheen in de *Acta Botanica Neerlandica* kreeg zij de Hugo de Vries-prijs van 1988. Na afloop van haar aanstelling bij BION zette zij als gastmedewerker haar werk binnen de werkgroep voort.

Zij is getrouwd met Martin Cox en heeft met hem twee kinderen.

Stellingen bij het proefschrift van Jeannette van der Sman, "Flooding resistance and life histories of short-lived floodplain herbs", 1-12-1992

1. Was het doel van MacArthur en Wilson (1967) nog om veel feiten te vervangen door één theorie, vijfentwintig jaar later is het nauwelijks meer mogelijk om voldoende onderzoeksgegevens aan te dragen ter ondersteuning van de vele bestaande theorieën.

2. In de discussie over de grootte- of leeftijdsafhankelijkheid van bloei (bijv. Lacey 1986) kan meer recht gedaan worden aan de plastische constructie van planten door het aantal en de verdeling van de samenstellende modules erbij te betrekken (dit proefschrift).

3. Begrippen als tolerantie (tolerance) en ontwijking (avoidance) zijn uiterst nuttig ter karakterisering van de reactie van planten op ongunstige milieufactoren, maar kunnen verwarring wekken wanneer het niveau van organisatie niet duidelijk van te voren wordt gedefinieerd (zie Levitt 1972; Fitter & Hay 1981; dit proefschrift).

4. Eén van de moeilijkste opgaven voor oecofysiologen is het vertalen van de resultaten van fysiologisch onderzoek, dat veelal wordt uitgevoerd met planten opgekweekt onder constante condities, naar een veldsituatie waarin geen enkele omgevingsfactor constant is.

5. Wij moeten de natuur trachten te begrijpen in plaats van haar te willen verklaren.

6. De strategieën "alles of niets" en "verdeel en heers" zijn dominant in zowel de biologische als de culturele realiteit.

7. Voor het oplossen van problemen van velerlei aard zijn zowel analyse als synthese nodig.

8. Het is een belangrijke taak voor onderzoekers en docenten in de biologie om de studenten meer te interesseren voor biologische processen en vraagstellingen dan voor het gebruik van geavanceerde apparatuur.

9. De toekomst vraagt van ons de bereidheid om hard te werken en sober te leven opdat welvaart plaats kan maken voor welzijn.

10. Voor mannen is het hebben van betaald werk een plicht, voor vrouwen een gunst.